406 MEDICAL GENERAL LABORATORY

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KOREA

TOKYO, HONSHU, JAPAN

OKINAWA

IWO JIMA

PHILIPPINE

SAIPAN

TINIAN
GUAM

ISLANDS

PROFESSIONAL SECTION

PREFACE

The continuance of war in Korea has introduced an increasing number of doctors of the United Nations to disease problems of the Orient, in addition to those military medical problems pertaining to care of the wounded. Although exotic diseases have seldom been a major concern, there have been certain conditions more or less peculiar to this area from the standpoint of etiology or prevalence.

This laboratory in fulfilling its designated mission "to supplement the epidemiologic, sanitary, and diagnostic services available in other medical department laboratories, and to investigate outbreaks of disease and conditions which affect, or may affect the health of persons .... of the command" has been a key position in the UN Medical Service. In addition to its role of scientific service to individuals, units, and staffs, the laboratory has continued its efforts in medical research activities. Any accomplishments in the latter field must pay acknowledgement to those partners in research - the patient, the doctor, and the many cooperating agencies.

The precedent of the past several years has established the reference value of previous issues of this annual publication. It is hoped that the present report will not only be of historic and reference value medically, but will also orient newly arriving doctors with diagnostic facilities available to them, with military problems which they will encounter in maintaining the combat strength of the command, and with the necessity of assisting in restoring and improving the health of civil populations.

The acknowledgement and thanks of this unit are also rendered to the many military and civilian individuals and agencies of the UN Command, of Japan, and the Republic of Korea.

With these purposes and thoughts the editors have submitted an anonymous representation of the efforts of many workers in the continuing war of man against disease.

R.L.H.

INTRODUCTION

The report which follows, describes the professional activities of a medical general laboratory in the communication zone of a combat theater. These activities may be divided into five categories; support of combat troops such as the blood bank activities, support of service troops by varied diagnostic services, assistance to preventive medicine officers by conducting epidemiologic investigations, research activities which are an extension of almost all other activities, and the training and assigning of laboratory officers to organizations throughout the theater. These activities, of necessity, vary with the local situation; requirements of the combat zone have the highest priority. The appearance of a disease, such as epidemic hemorrhagic fever, requires directing maximum efforts towards investigating this problem with a consequent decrease in emphasis on the study of other conditions.

Although no effort has been made to correlate in chronological form the various laboratory activities and investigations with changes in the combat situation, it is believed this report will be of value to those interested in military laboratory functions because it demonstrates the types of problems which may be encountered. Obviously, a great deal of material has been collected which will require much additional study before final conclusions can be reached.

There have been personnel changes which are increasing in tempo. Nevertheless, a most gratifying continuity of effort has been maintained in research and planning. This speaks well for the planning and training programs of the Laboratory and Allied Sciences Branch and the Research and Development Board of the Office of the Surgeon General, as well as the Army Medical Service Graduate School, from which organization much valuable advice and assistance has been received.

Finally, as indicated in many places, this report, originating in the 406th Medical General Laboratory, is in large part an acknowledgement of the contribution of and a tribute to the many laboratory officers in the entire theater whose interest, cooperation, and efforts were of fundamental importance to the work presented herein.

R.P.M.

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EPIDEMIOLOGY SECTION

The Epidemiology Section was activated as a part of the laboratory headquarters in January 1951, with one medical officer engaged full time in epidemiological activities. The purpose of the section has been to conduct, in collaboration with the technical departments concerned, epidemiological investigations on special problems of interest. This section has not engaged in operative preventive medicine, but has rather attempted to provide the laboratory and the preventive medicine consultants within the theater, epidemiological information of specific and detailed nature on certain disease problems which it has been called upon to investigate. The following report makes no attempt to discuss the epidemiological aspects of all the diseases which concerned the laboratory or medical personnel of the Far East Command during 1951. It rather presents brief accounts of a few of the problems which were studied and thereby reflects somewhat the more pressing preventive medicine problems facing medical personnel in the command during 1951.

SMALLPOX: Incidence in Japan and Korea, 1945-1951 - The incidence of smallpox among allied personnel in the Far East Command has been marked by two distinct periods of high incidence, one in the winter of 1945-1946 and another in the winter of 1950-1951. Both of these outbreaks arose when large numbers of allied troops suddenly became associated with the population of Korea. Morbidity statistics for the native population of Korea are highly inaccurate. However, widespread endemicity in Korea was well recognized by the Japanese for many years (1). Repeated severe urban epidemics stimulated limited vaccination programs sufficient to bring these epidemics under temporary control but adequate nationwide vaccination programs were never accomplished. During late 1945, after the arrival of American occupation personnel, 23 outbreaks of smallpox in the Korean population were reported by public health workers. In Japan, on the other hand, a nationwide vaccination program has been in operation for several decades, so that the national level of immunity in the native population of Japan has usually been high enough to prevent all but sporadic occurrences and occasional small limited outbreaks (2). The one notable exception was the post-surrender epidemic of nearly 18,000 cases in Japan during the Spring of 1946 (3). The reported cases of smallpox among occupation personnel in Japan and Korea and among the indigenous population of Japan during the occupation years are shown in Table I.

Table I. Reported Cases of Smallpox in Occupation and Other UN Personnel in Japan and Korea (exclusive of ROK's) and in Japanese Nationals,* 1945-1951

| | Occupation & UN Personnel | | Japanese |
	Japan	Korea	Nationals
1945	0	24	1,614
1946	17	0	17,800
1947	0	0	391
1948	0	1	29
1949	0	0	124
1950	0	5	5
1951	1	34	86

* Annual Summaries, Public Health and Welfare
in Japan, PH&W, GGQ, SCAP, 1948-1950

Within two months after the arrival of American troops in Korea in the fall of 1945, American cases of smallpox began to appear. Between October and December, a total of 24 cases had occurred. Immediately after the recognition of the first case during the last week of October 1945, all military and civilian personnel in Korea were vaccinated. The command responsibility for immunization was emphasized and particular attention was given to avoid the introduction of unprotected replacements into Korea. Nine cases occurred in November and fourteen in December of 1945. The

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last case in U. S. occupational personnel had its onset on 26 December 1945, just two months after the first case appeared. It should be emphasized that these 24 American cases did not occur in the same area and did not as a group represent a contagious chain. On the contrary, they arose from such distant and scattered points as Seoul and Won Ju in the north and Mokpo and Pusan in the south. The highly virulent form of smallpox which was encountered in Korea at that time is emphasized by the fact that at least 12 of these cases died. Seven of the fatalities were due to the purpura variolosa variety of smallpox and three to variola hemorrhagica. The other two were in cases of classical smallpox. Only two cases of mild or varioloid smallpox occurred; these were in individuals who had visible evidence of a previous vaccinia reaction. None of the other 22 cases had ever had primary vaccinia reactions prior to induction and only two of these 22 developed vaccinia reactions from army vaccination prior to coming overseas. It is noteworthy that a number of these persons who had never had primary takes had had one or more army vaccinations against smallpox with reactions recorded as immune.

In January 1946, close on the heels of the outbreak in Korea, 17 cases of smallpox occurred in U. S. occupation personnel in Kyushu, the southernmost island of Japan (4). It is of interest to note that these cases occurred in one of Japan's busiest port areas, in fact the port most commonly utilized by vessels plying between Japan and the southern ports of Korea. In this outbreak there were 10 deaths, 8 due to purpura variolosa and two to variola hemorrhagica. The fatality rate in these two forms of smallpox was again 100%. During 1946, vaccination requirements for occupation personnel in Korea were boosted to every 6 months and within 60 days of departure from Korea, and yearly vaccination was established in Japan. These schedules have been maintained since that time. It was recognized that endemicity among the native population of Korea and possibly of certain parts of Japan immediately following the end of the war was sufficiently high to produce outbreaks in occupation personnel if even a fraction of a per cent of American troops were inadequately immunized. Hence, great emphasis was placed not only upon complete coverage but on accurate interpretation of reactions and repeated vaccinations in all cases of doubt.

From 1946 to early 1948 sporadic reports of smallpox cases and deaths from all parts of Korea indicated that the disease continued unabated in the native population. In addition to internal spread, smallpox was probably being introduced by refugees who were streaming to South Korea from China and North Korea at the rate of one to four thousand per month.

In November 1948, a large outbreak of smallpox was reported among civilians in Seoul, Korea. This probably represented a true increase in incidence although previous reports were too vague to be certain. In November, 163 cases with 51 deaths and in December, 198 cases were reported. Considerable public interest in the epidemic seems to have arisen as indicated by the fact that the entire city of Seoul was canvassed by teams of Korean physicians, medical students and police and occupation public health advisors, and all cases were transferred to the city's contagious disease hospital. This epidemic was not confined to Seoul but rather represented a period of Korea-wide high endemicity lasting well into May of 1949.

There were no cases of smallpox among occupation forces in the Far East from the spring of 1946 until late 1948 when one non-fatal case appeared in an air force sergeant in Korea in December. He had been missed during the 1948 vaccination program in Korea and had not been vaccinated since November 1947. The reaction at that time is not known. Following this case, no smallpox occurred among occupation personnel in the Far East Command until after the outbreak of the current Korean conflict.

Meanwhile, during all of 1948, and throughout the latter half of 1949, smallpox was also rare in Japanese. Only 29 cases in 1948 and 124 cases in 1949 were reported in Japan. The 1949 cases were concentrated into two port areas of southern Japan and most cases occurred between March and June. A nationwide vaccination program was initiated in 1949 and completed in 1950, and is reported to have covered "nearly the entire population" (5). This seems to be supported by the fact that only 5 cases occurred in Japan during 1950 in spite of the sudden influx from Korea of smuggled refugees and UN returnees and evacuees, among whom were a number of smallpox cases.

Thus, despite continued high endemicity among the native population of Korea and sporadic occurrences in Japan, smallpox was effectively eradicated from Americans in the Far East Command, within four months after its initial appearance in October, 1945. The disease was, for all practical purposes, non-existent in military personnel from early 1946 to the fall of 1950. Then at the beginning of the Korean war, many American troops, previously unexposed to the orient, made sudden and widespread contact with the Korean population. The first cases appeared in October, 1950, and they continued to occur sporadically among UN forces in Korea until April, 1951. One isolated case occurred in July, 1951. A total of 39 cases were contracted in Korea by UN forces, exclusive of Korean troops, during the first year of the war. During that same period one fatal case occurred in a civilian who never left Japan. However, epidemiological investigation strongly suggested that the ultimate source of infection in this case was Korea (Smallpox Outbreak in Kobe, below). Only 14 of these cases were in U. S. army personnel, more than half the cases having appeared in other American and other UN troops. The distribution of these cases by month and organization in the Far East Command since the beginning of the Korean conflict is shown in Table II. Out of the total of 40 cases, there were 14 deaths. Autopsy material on nine of these deaths was reviewed by the Department of Pathology of this laboratory. The diagnosis of classical smallpox was made in three cases, of variola hemorrhagica in three cases and of purpura variolosa in four cases. The case fatality rate for the latter two forms was again 100%. A discussion of the pathologic findings in fatal smallpox is presented in the report of the Department of Pathology. A brief account of the clinical findings in purpura variolosa is presented below. Variola virus was isolated from most of the cases on which material was submitted, and it was retrieved from whole blood, serum, skin, vesicular and bullous fluid and kidney. (See report of Department of Virus and Rickettsial Diseases).

It should be emphasized that in all cases with available immunization records vaccination had not been carried out according to existing regulations. Either there was no evidence of a previous primary vaccinia reaction or the latest vaccinoid or immune reaction had been recorded well over a year preceding onset. No fatal cases occurred in persons successfully vaccinated within three years of onset. In several instances, including one fatal cases, an immune reaction was recorded within two years, but on questioning the patients, it was found that there had been no reaction whatsoever at the time of recording. This fatal case, a civilian in a fixed installation in Japan, had never experienced a primary vaccinia reaction in his life,(see report of Kobe Outbreak of Smallpox).

The incidence of smallpox in Japan rose to 86 in 1951. All cases occurred between February and July with a peak of 30 cases in March (6). The peak month in UN Personnel in Korea was likewise in March. The geographical distribution of cases in Japan is interesting in that 72 of the cases were reported from only four prefectures of southwestern Japan, whereas only one case was reported from the Tokyo-Yokohama area. Japanese health officers have considerable evidence to indicate that much of this smallpox was introduced from Korea. Cases and outbreaks were usually traceable to ports and fishing villages engaged in sea traffic with Korea. For example, one outbreak of 13 cases in Yamaguchi Prefecture was traced directly to a Japanese fisherman who contracted smallpox during a series of undercover junkets to Korea (6). A comparable situation in Kobe is described in detail below. UN medical personnel in Korea stated that smallpox was commonly seen among Korean nationals in 1951, however morbidity statistics were not compiled.

Twenty-two cases of smallpox with one death occurred in North Korean and Chinese prisoners of war since the beginning of hostilities. The peak incidence also was in March 1951.

Smallpox Outbreak in Kobe - During the second week of February 1951, nine clinically confirmed cases of smallpox suddenly appeared in Kobe, Japan. All nine cases had dates of onset from 9 to 12 February. Eight of the nine cases were employees of the Kobe Laundry and Dry Cleaning Plant, an occupation establishment. The other case

Table II.   Smallpox in UN Personnel (exclusive of ROK) FEC – July 1950 to December 1951

Cases/Deaths

	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Cases	Deaths
U.S. FORCES																				
US Army				1/1		1/1	2/1	2/0	6/1	2/0									14	4
US Air Force					1/0					1/0			1/0						3	0
USMC				2/0			1/1	4/1											7	2
US Navy							1/0	1/1	2/0										4	2
DAC								1/1											1	1
US Mer. Seamen									2/1										2	1
Total US				3/1	1/0	1/1	4/3	8/3	10/2	3/0			1/0						31	10
OTHER UN TROOPS																				
United Kingdom									1/0										1	0
Canada									1/0										1	0
New Zealand							1/1												1	1
Philippines								1/0	1/0										2	0
Turkey									3/3	1/0									4	3
TOTAL OTHER UN							1/1	1/0	6/3	1/0									9	4
TOTAL CASES				3	1	1	5	9	16	4			1						40	
TOTAL DEATHS				1	0	1	4	3	5	0			0							14

was in a Japanese who was not associated in any detectable way with the laundry outbreak.

Of the 8 cases in the laundry and dry-cleaning plant, 7 were Japanese employees and one was an American civilian manager. This American case was mentioned in the preceding discussion. The cleaning establishment consisted of two adjacent but separate buildings, a laundry and a dry-cleaning plant. Six cases arose in laundry employees and two in the dry-cleaning plant. The cases are tabulated by order of onset in Table III, showing certain data discussed below.

A common human source in the form of an earlier unrecognized case, and a fomite source were originally considered. Neither a common meeting point nor a previously unrecognized case could be found. This search included review of employees' absentee records and attempts to discover a mild or unrecognized case. The fomite to which most and probably all patients could have been exposed was incoming salvage material. Salvage was being received at that time from several points in southwestern Japan through a Kobe port reclamation and salvage section and from a marine air base. The marine corps material was being received directly from land units in Korea. Since cases of smallpox were occurring among UN troops in Korea at that time, it is quite possible that clothing and equipment from these or from mild unrecognized cases could have reached salvage piles either from parent units or from receiving hospitals. Virus could have persisted in a viable state in crusts and dried secretions on this material and its distribution would have been enhanced by the clouds of dust arising from such items as damaged sleeping bags.

The absence of cases in the Reclamation and Salvage Section personnel who sorted all material before it reached laundry personnel, suggested that the specific source might have been through the Marine Air Base. The marine material was carried directly from the air field to the laundry with minimal handling.

It was not possible to ascertain which shipment of salvage material was responsible for exposing the cases. However, it was interesting to find that one shipment of sleeping bags was received on approximately 27 January and was sent to the laundry building by mistake. It was unpacked and when the mistake was discovered it was repacked and carried to the dry cleaning plant for processing. The entire maneuver required about three days. The significance of this observation is a matter of speculation; however, this was the only shipment of salvage material which actually entered both buildings. Furthermore, the interval between this episode and the outbreak is about two weeks, a plausible incubation period for smallpox.

An investigation of smallpox vaccination records of patients showed that the most recent vaccination had been accomplished in May or June of 1949. The annual universal vaccination program for indigenous employees at Kobe originally scheduled for November 1950, had been omitted for administrative reasons. The one fatal Japanese case had not been vaccinated in 35 years. The fatal American case had been vaccinated in May 1959; his immunization register showed an entry of immune reaction at that time. However, questioning of the patient revealed that he had had no reaction whatsoever and furthermore had no evidence of a previous vaccinia reaction. Immediately after the appearance of the first case, a universal vaccination program for both Japanese and occupation personnel of the entire city of Kobe was instituted.

Purpura Variolosa - Clinically, the most striking aspect of the smallpox seen arising from Korea has been the high incidence of variola hemorrhagica and so-called purpura variolosa. As mentioned previously, these forms were also seen in Korea in 1945. Variola hemorrhagica was usually readily recognized as a form of smallpox since characteristic vesicles and pustules appeared at about the same time as in classical smallpox. On the other hand, because of the absence of typical variola lesions in purpura variolosa it is often not considered even in areas where smallpox is regularly occurring.

Table III. Smallpox Cases - Kobe, 1951

Case No.	Name	Nationality	Onset Date	Occupation	Date and Reaction of Last Vaccination	Clinical Type*	Outcome
1.	K.K.	Japanese	9 Feb 51	Laundry Supply Officer	1947 - Unknown	Typical - mild	Recovered
2.	K. I.	Japanese	9 Feb 51	Dry Cleaning Plant Superintendent	May 1949 - Unknown	Typical - mild	Recovered
3.	M. K.	Japanese	10 Feb 51	Laundry Ironer	May 1949 - Immune	Typical - mild	Recovered
4.	M.	Japanese	10 Feb 51	Laundry Ironer	Unknown	Typical - mild	Recovered
5.	C. B.	American	10 Feb 51	Laundry Manager	May 1949 - No reaction	Typical - severe	Fatal
6.	K. W.	Japanese	11 Feb 51	Dry Cleaning Plant Receiver	Unknown	Typical - mild	Recovered
7.	S.	Japanese	12 Feb 51	Laundry Extractor	Not vaccinated during past 35 yrs.	V.H.	Fatal
8.	K. O.	Japanese	12 Feb 51	Laundry Folder	May 1949 - Unknown	Typical - severe	Recovered
9.	K. E.	Japanese	12 Feb 51	Pimp	Reports immune	Typical - mild	Recovered

* typical - classical vesiculo-pustular variola

V. H. - variola hemorrhagica

Of the 40 cases of smallpox reported in UN Personnel since the beginning of the Korean war, 4 cases have been of the purpura variolosa variety. As in past experience, all cases were fatal. In three out of the four cases, a clinical diagnosis of smallpox was not made. Only after autopsy findings and smallpox virus isolations had demonstrated the true nature of the disease did medical personnel become alert to this entity, and the fourth case was readily recognized before death. These facts are all the more important since the application of protective measures was delayed or even omitted because of the failure to recognize cases before death. Because of this year's experience with the pitfalls which are encountered in dealing with purpura variolosa, a brief clinical account seems warranted here.

The first two or three days of disease usually presented symptoms and signs characteristic of almost any infectious disease. Severe backache was the only symptom which suggested the prodrome of smallpox. The first specific physical manifestation was a marked, generalized, dusky red, blanching flush on the second or third day. Superimposed upon this, there was in some cases a discrete erythematous maculo-papular eruption. In others a morbilliform rash was noted. Usually, within a matter of hours hemorrhages appeared in the sclerae and in the oral mucosa and petechiae began to appear on the skin of the trunk. Edema of the face, especially striking about the eyes, was usually seen. Gross hematuria, hemoptysis, hematemesis and bloody stools often appeared within a day after the appearance of skin lesions. The total white blood count rose rapidly to 20,000 - 40,000 per cu. mm. and a leukemoid picture was noted. Immature members of the erythrocytic series were seen in the peripheral blood in all cases. As the hemorrhagic process advanced, it involved the entire skin surface producing large confluent purpuric blotches superimposed upon a marked erythema. The sclerae showed massive confluent hemorrhage completely obscuring the whites of the eyes. The bleeding time was markedly prolonged and injection sites bled freely and continuously. Bullae containing serosanguinous fluid developed, especially on the extremities, and large sections of epidermis were sloughed with ease. By the fourth to sixth day of disease temperature usually fell to normal or below and a shock-like picture developed. Whole blood transfusions had little effect. Respiratory embarrassment was marked and was apparently due to both severe bronchial involvement and pulmonary edema and hemorrhage. Death followed massive hemorrhages from the orifices in one case, but in the others it could be attributed to no one particular manifestion of the syndrome. Death occurred on the sixth to eighth day of disease.

As might be expected, a variety of diseases characterized by hemorrhagic diathesis were suspected clinically. Diagnoses of meningococcemia, epidemic typhus, acute myeloid leukemia, toxic non-thrombocytopenic purpura and epidemic hemorrhagic fever were entertained. In fact the earliest interest in epidemic hemorrhagic fever was aroused by certain similarities between the cases of purpura variolosa and the available clinical descriptions of epidemic hemorrhagic fever by Japanese workers. Later, when true epidemic hemorrhagic fever began to appear in UN troops, it was realized that the severe and fatal cases of this disease did bear a certain striking resemblance to purpura variolosa and would probably present the greatest difficulty in differential diagnosis.

Perhaps the most helpful distinguishing features between purpura variolosa and epidemic hemorrhagic fever are the skin manifestations. Although epidemic hemorrhagic fever almost always shows a facial and shoulder flush, and frequently shows scattered small petechiae over the upper trunk, it has never shown the generalized deep flush or widespread, extensive, and later confluent ecchymoses seen in purpura variolosa. Bulla formation and massive sloughing of the skin have not been seen in epidemic hemorrhagic fever. Together with these findings epidemiologic features are often helpful in distinguishing the two. Epidemic hemorrhagic fever is thus far known to occur only in individuals with outdoor ground exposure and usually will be seen during already recognized outbreaks in which many milder cases are appearing. Smallpox among American troops is usually sporadic and is likely to be more common in indoor and urban environments. Finally, single cases of smallpox are rare. Purpura variolosa nearly always occurs in association with easily recognized cases of classical smallpox.

JAPANESE B ENCEPHALITIS: Japanese Nationals - In Japan in 1951, the Ministry of Health reported 2152 cases of Japanese B Encephalitis with 945 deaths (6) or about half the cases reported in 1950. Tokyo-to showed even a more striking reduction of cases during this year. While 1182 cases with 321 deaths occurred in Tokyo in the summer of 1950, this year only 181 clinically confirmed cases and 42 deaths were reported. The first case in Tokyo in 1951 had its onset on 16 July and the last on 22 October with a peak of 49 cases during the week ending September 15. It is of interest that not only was the epidemic in Tokyo of much smaller proportions in 1951, but also that it reached its peak just one month later than did the 1950 outbreak. Comparison of 1950 and 1951 epidemic curves is shown in Figure 1. The time distribution of fatal cases roughly parallels the epidemic curve. The National Institute of Health of Japan performed complement fixation tests on serial sera submitted on these cases and reported either a four-fold titer rise or a single titer of 1:16 or over in 99 non-fatal cases. The distribution of these serologically confirmed cases also parallels the epidemic curve. Only one fatal case was serologically confirmed. No data are available on virus isolation or histological confirmation of fatal cases. Clinically confirmed cases, deaths and serologically confirmed cases are shown by week of onset in Figure 2. A tabulation of cases and deaths due to Japanese B Encephalitis during selected previous years is shown in Table IV. Figures for 1924 and 1935, the two highest prewar epidemic years, are given for comparison.

Table IV. Japanese B Encephalitis, Japan, 1924 - 1951

		TOTAL	JAPAN			Tokyo-To			
			Case Fatality Rate	Peak Week				Case Fatality Rate	Peak Week
	Cases	Deaths				Cases	Deaths		
1924	6949	4164	56%						
1935	5370	2264	42%						
1946	150 (appr)	-	-						
1947	259	133	51%						
1948	7208	2621	36%	Aug - 3d wk.		1969	527	26%	Aug. 2d wk.
1949	1294	1182	-	Sept		216	86	35%	Sept. 2d wk.
1950	5182	2440	47%	Aug		1182	321	27%	Aug. 3d wk.
1951	2152	945	44%	Sept - 3d wk.		181	42	23%	Sept. 3d wk.

Occupation Personnel in Japan - Only 11 cases of encephalitis and no deaths were reported in occupation personnel in Japan in 1951, and it is highly doubtful that all of these represented infection with the JBE virus. Onsets by month were as follows: April, 1; June, 3; September, 4; October, 2; and November, 1. The submission of serum samples was highly inadequate; paired sera were received on only seven cases. Negative CF results were obtained on six of the seven, but convalescent sera were obtained after the 21st day of disease in only three. The only Japan case confirmed serologically in 1951 was a U. S. Army case from the Osaka area with onset 13 September. This individual developed a 16-fold titer rise in CF antibodies during the course of illness. In view of past and current experience with this disease by both Japanese and American workers, it is highly doubtful that any of the cases reported in occupation personnel before September of this year were in fact infections with the JBE virus. Thus the total cases probably do not exceed seven. (See report of the Department of Virus and Rickettsial Diseases).

Little is known of the sources and modes of transmission of Japanese B Encephalitis except that in Japan the mosquito, Culex tritaeniorhynchus, is naturally infected during certain pre-epidemic and epidemic periods. (See Report of Department of Entomology). There is strong evidence to indicate that this mosquito serves as a vector of human and mammalian infection in Japan and little or no evidence to incriminate other species. Hence, in the search for factors contributing to the variation in time
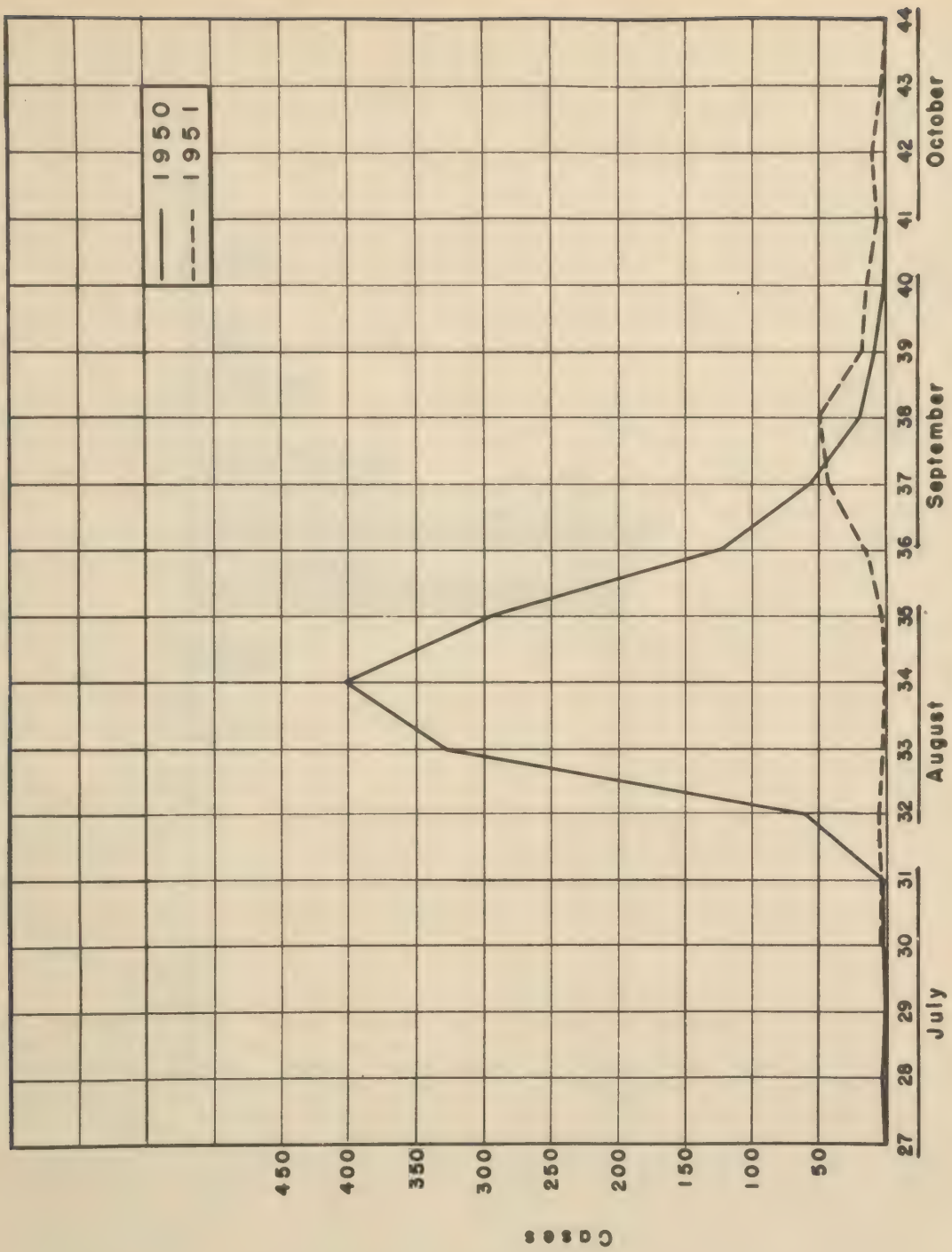
Figure 1. Japanese B Encephalitis - Tokyo-to, 1950-51 By Week Of Onset
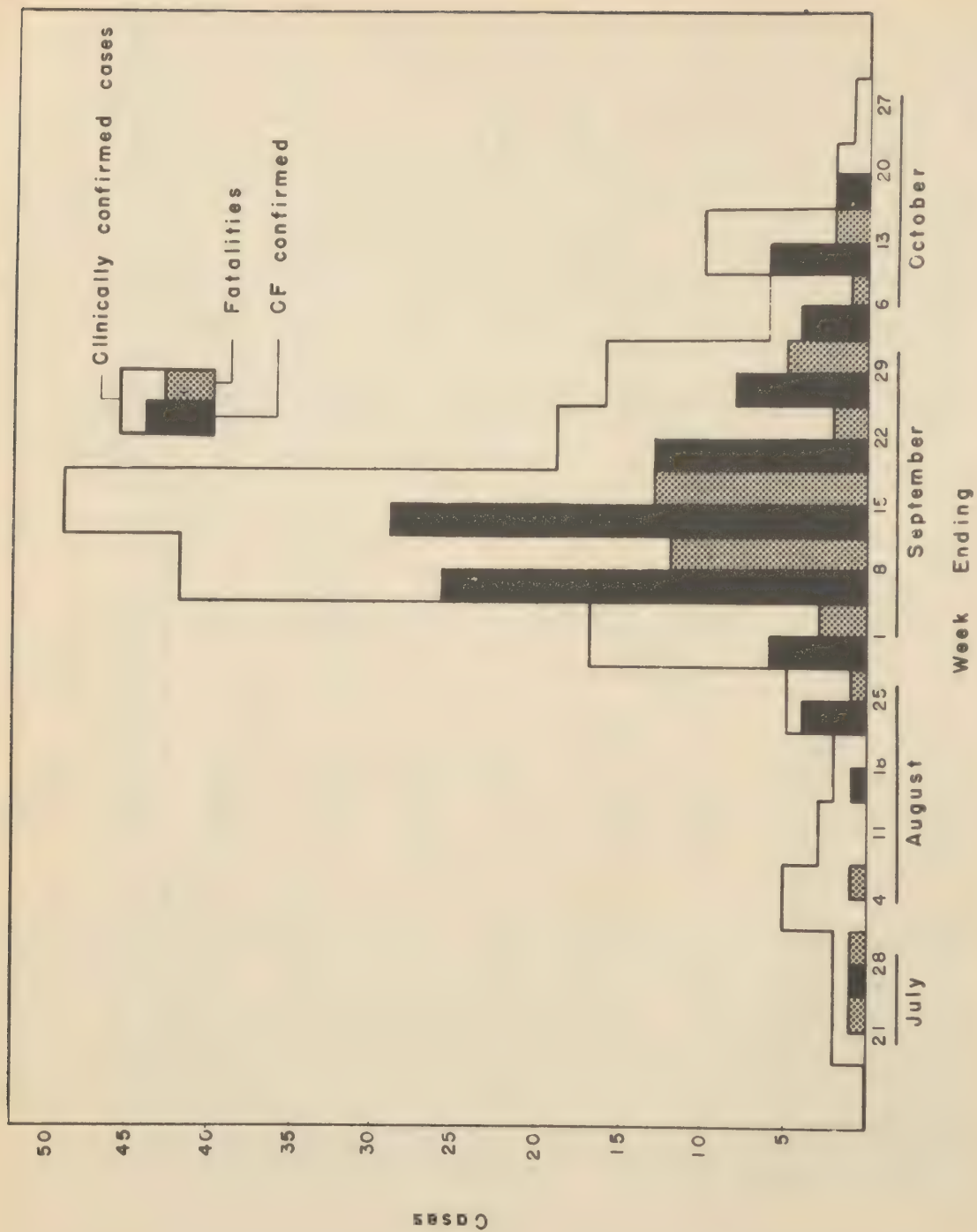
Figure 2. Japanese B Encephalitis – Tokyo-to, 1951 by Week Ending

and incidence of human disease from season to season, ecological factors influencing the prevalence and behavior of this mosquito have been strongly considered. Detailed studies attempting to correlate meterological data with both mosquito populations and the incidence of human and mammalian encephalitis in Japan are presented in the report of the Department of Entomology. Although earlier impressions of Japanese workers (7) and observations on the Tokyo epidemics of 1950 and 1951 suggested that a higher incidence of Japanese B Encephalitis followed periods of high temperature and low rainfall, analysis of weather data over a period of several years now indicates that no definite correlation can be made. Apart from weather considerations, however, it is interesting to note that C. tritaeniorhynchus horse bait trap collections and human biting data both indicated extremely low mosquito populations in Tokyo during the summer of 1951. Also, the sharp population peak usually seen in this species was absent this year. It should be emphasized, however, that the virus of JBE was again isolated from C. tritaeniorhynchus lots, in spite of the fact that only small numbers of mosquitoes were available for testing.

Japanese B Encephalitis in Korea - No data are available on the occurrence of encephalitis in native Koreans in 1951. Unofficial communications and observations indicated, however, that if it was occurring, it was indeed rare. Twenty-one cases of suspect encephalitis and two deaths were reported among UN Personnel for the year, but as in the case of Japan, onsets were widely scattered throughout the year, and many certainly do not represent infections with the JBE virus. Onsets were reported by month as follows: February, 3; April, 3; May, 1; July, 1; August, 6; September, 6; and November, 1. The two deaths occurred in April and September. Negative CF results were obtained in 12 serum series but only five of these series extended past the 21st day of illness. The April death was histopathologically encephalitis, etiology undetermined. The histologic findings were inconsistent with Japanese B Encephalitis. Only two confirmed cases of JBE occurred in Korea this year. Both were in U. S. Army personnel and both had onsets in September. In one, a four-fold titer rise in CF antibodies was obtained, and in the other, a fatal case, JBE virus was isolated from the brain and the histologic findings were those of Japanese B Encephalitis.

Okinawan Natives - During midsummer of 1951 a small outbreak of encephalitis occurred in Okinawan natives. Forty-five cases were reported. Dates of onset were indicated in 31 cases, ranging from 21 June to 2 September. Twenty-six of these 31 had onsets in July with a sharp peak of 15 cases during the week ending 21 July. Most cases were in males under 20 years of age. Serum pairs were submitted to the 406th Medical General Laboratory on very few cases. Two tube titer rises or a single titer of over 1:16 in the complement fixation test were found in only five instances. Five cases showed suspicious titers and three were still negative after the 30th day of disease.

Occupation Personnel in Okinawa - Suspect encephalitis was reported in occupation personnel in Okinawa from mid-March through mid-November. Thirty-four cases and one death were reported for the year with onsets clustered in two groups: one group of 18 cases with onsets from 18 March to 12 June with a peak in mid-May and another group of 16 with onsets from 3 July to 17 November with a peak in the third week in July, coincident with the peak in the native population. Serial sera extending at least to the 35th day of disease were examined on 10 of the earlier cases with universally negative results. Clinical records indicate that many of these earlier cases reported as encephalitis did not show convincing evidence of central nervous system infection. On the other hand 4 cases of laboratory confirmed JBE occurred from July to November. Three cases showed diagnostic titer rises in serum pairs and JBE virus was isolated from the brain of the fatal case with onset on 6 November. This case is of special interest since it demonstrates for the second time the late fall occurrence of JBE in Okinawa. One case with onset on 17 October 1950 was also confirmed serologically by this laboratory (8).

Summary - During 1951, Japanese B Encephalitis in UN personnel in the FEC has been noteworthy because of the paucity of cases of clinical encephalitis and the infrequency with which infection with the JBE virus has been demonstrated. Only 66

cases of clinically diagnosed encephalitis were reported for the entire theatre and at least 27 of these were cases that would usually be considered out of season. All attempts at laboratory confirmation in this out of season group were negative. Out of the remaining 39 cases only 7 showed laboratory evidence of JBE infection. In Japan one case was confirmed serologically. In Korea, one case was confirmed serologically and one fatal case was confirmed histologically and by virus isolation. In Okinawa, three cases were confirmed serologically and one fatal case was confirmed by virus isolation. This gives a total of 7 confirmed cases including 2 confirmed deaths due to Japanese B Encephalitis in the Far East Command in 1951.

PNEUMONITIS IN KOBE: During early February 1951, an outbreak of 37 cases of an acute respiratory infection characterized by fever, chills, malaise, myalgia, headache, anorexia, cough, chest pain, and a patchy pneumonitis by x-ray, appeared in hospital personnel and patients in a U. S. Army Hospital in Kobe, Japan. Retrobulbar pain, stiff neck, nausea and vomiting as well as mild hemoptysis were frequently noted. Hepatomegaly, pulmonary signs and splenomegaly were less frequent. One notable finding was the frequent appearance of tiny telangiectasis on the distal parts of the extremities. Peripheral blood studies were not remarkable except for occasional mild eosinophilia.

Onsets of disease were between 30 January and 9 February with a peak incidence of eight cases on 4 February. Nineteen were patients already hospitalized for other causes, 17 were nurses, doctors and other hospital personnel, and one had been a visitor. Only 7 other cases of acute respiratory disease were hospitalized during this period, so that the outbreak represented a distinct rise in respiratory disease admissions for that hospital. Epidemiological investigation revealed that all patients and nearly all employees had been on the second floor of the hospital during late January. No primary human source could be found and no evidence of secondary cases occurred. Hence, an airborne agent not readily transmitted by active human cases was suspected.

Because a pair of parakeets had been kept on the second floor of the hospital during the postulated exposure period, psittacosis was originally suspected. However, pathological examination of the birds gave no evidence of infection, and attempts to isolate an infectious agent were unsuccessful. No new parakeets have been imported to Japan within six years and all birds are now derived from a common stock in southwest Japan. Furthermore, psittacosis is not known to occur in Japan.

Q fever was considered, but no source of contact with animal reservoirs or their products could be found. Cold agglutinins were negative. Exhaustive attempts to establish a specific agent as the cause of the outbreak yielded negative results. The material tested and the results are presented in the Report of the Department of Virus and Rickettsial Diseases. Although a known etiologic agent was not identified, the homogeneous clinical picture and the striking epidemiological features of the outbreak led most observers to conclude that a specific infectious agent was responsible.

INFECTIOUS HEPATITIS: Interest in the epidemiology of this disease in Japan and Korea has been greatly stimulated by the marked increase in incidence in both areas since the late months of 1950. This led to a detailed investigation of one small area of high endemicity in an attempt to define certain of the ecological features of the disease in local situations.

Air Force and Army personnel at an air base in northern Japan suffered a rate of 13.2 per 1000 per year, between July 1950 and March 1951, reaching a peak in December of 1950. Army personnel, consisting entirely of anti-aircraft artillery troops, experienced a rate of 27 per 1000 per year, while air force personnel suffered a rate of 7 per 1000 per annum, only slightly higher than the usual rates for all troops in Japan during the earlier occupation years. Cases were confined to enlisted military personnel, although officers and dependents were present on the base in numbers. Similarly, none of the numerous Japanese employees had recognized clinical disease. Clerical, mess, and medical personnel had almost no cases. Detailed investigation of the area revealed that the only essential differences in environmental conditions between the Army and

the Air Force troops were in messing facilities and actual duties performed. Permanent messes were of the same type for both army and air force. However, anti-aircraft artillery gun crews often messed under field conditions where sterilization of utensils was distinctly inadequate. The AAA gun pits occupied positions formerly devoted to truck farms which had been treated with night soil. Routine duties of gun and service crews required contact with open soil and with unsanitary field conditions much more than was required of air force personnel.

Fraternization with the indigenous population of the two adjacent villages was common and equal in both army and air force personnel and in clerical as well as tactical soldiers. Fraternization was uncommon among officers and enlisted men with dependents. Although association was greater in the early months of 1950 than after the beginning of the Korean conflict, hepatitis did not begin to appear in notable numbers in American personnel until after July, 1950.

Discussion with local Japanese physicians and examination of hospital records in a village in direct contact with the base and in a small isolated village about ten miles from the base revealed that simple or catarrhal jaundice was fairly common among Japanese children between the ages of five and ten years, and during 1950, showed a distinct seasonal peak in October. The two villages showed essentially parallel findings. Rough calculations indicated that the morbidity rate of icteric hepatitis in that part of Japan was about the same as the overall morbidity rate for infectious hepatitis among occupation personnel in Japan. Since non-icteric hepatitis is frequent among children, the incidence of infection with hepatitis virus among Japanese may be much higher than among Americans.

SCRUB TYPHUS: Scrub typhus has long been recognized in Japan as an endemic disease in several of the river valleys on the western coast of northern Honshu. In October and November of 1948, 24 cases of the disease appeared in American troops bivouacked on the slopes of Mt. Fuji (9), about 50 miles west of Tokyo, thus establishing the existence of a hitherto unknown endemic area. Current investigations strongly suggest that some form of mite-borne typhus is endemic on the Izu-Shichito island chain off the east coast of Japan (10). However, until 1951 there were no indications that scrub typhus might occur in Korea. Japanese workers, familiar with the disease in their homeland, had not encountered it during some 40 years of Korean experience and no cases had been reported in American occupation personnel in Korea during the post-war period.

The first indication that scrub typhus may be indigenous to Korea came with the reporting in the summer and fall of 1951 of six cases among UN personnel on duty there. Five of the cases appeared in British Commonwealth combat soldiers from June to August. These cases all arose from the general vicinity of the Imjin River valley in west-central Korea. The first two cases with onsets of 8 and 10 June were from the same infantry battalion. Subsequent cases with onsets on 14 July, and 5 and 16 August were from scattered units within the same general area. All cases were in individuals in front line units who were frequently exposed to the ground. The reported clinical pictures varied considerably. Eschar was reported in three cases and rash in two. Material for rickettsial isolation was not received on any of these cases, but sera were tested for agglutinins in two laboratories. The results were somewhat varied but indicated either definite OXK titer rises or markedly elevated single serum titers in all cases. (See reports of the Department of Bacteriology and the Department of Virus and Rickettsial Diseases).

After the first two cases were reported in June, two officers from this laboratory visited Korea for the purpose of investigating the occurrence of this disease there. No evidence of previously unrecognized cases of this disease among other groups of UN troops or among Korean natives could be found. Units subsequently occupying the same position as that occupied by the unit producing the first cases were carefully observed, but no new cases appeared. Serum pairs were collected on 16 members of the unit having cases in June and were tested for OXK agglutinins. None of these showed

evidence of having acquired an inapparent infection during July or August. Trombiculid mites were found very commonly on wild rodents collected from several areas in west-central Korea. At the present time, no attempts have been made to isolate rickettsia from rodents or mites.

The sixth case of scrub typhus occurred in November in a USMC sergeant who was stationed near Masan, on the Southern coast of Korea, during his period of exposure. The clinical summary in this case is presented below:

The onset of illness on 27 November 1951, was sudden with chills and fever. A rash was first noted on the chest on the third day of illness, becoming generalized over the next 24 hours. At this time a crusted lesion was present on the left shoulder. On admission on the fifth day a generalized rash, eschar, fever, and headache were present. Chloromycetin was started on the 7th day of illness with dramatic improvement of symptoms. The patient was afebrile after 36 hours. Proteus OXK agglutinins were negative on the 7th and 13th day of illness rising to 1:80 by the 18th day.

These six cases could serve only as suggestive evidence of the existence of scrub typhus in two widely separate parts of Korea. In view of the large numbers of UN troops living in intimate contact with the ground and ground cover in certain parts of Korea throughout the summer and fall of 1950 and 1951, it seems unlikely that scrub typhus is highly endemic in any of these areas. By the same token the lack of characteristic localized outbreaks among Japanese troops stationed in Korea for many years would militate against any widespread or heavy endemicity. However, scrub typhus has frequently cropped up in localities which were previously inhabited and considered free of the disease. It has also occurred under a great variety of climatic and ecological conditions. These facts are emphasized by the recent discovery of new endemic areas in Japan. It follows that localized endemicity could have gone long unrecognized in Korea and the present evidence for this deserves further investigation.

MALARIA: Malaria has long been recognized as a highly endemic disease in southern Korea (1) whereas in Japan, it has within recent times been a disease of low incidence (2). The only endemic area of importance in Japan is in Shiga prefecture in the lowlands surrounding Lake Biwa in central Honshu. In 1950, only 1017 cases were reported (5) and in 1951, only 478 cases occurred in the entire country (6). Similarly, almost no malaria has been contracted in Japan by occupation personnel throughout the past six years, whereas in the 1945-49 occupation of Korea, malaria appeared in considerable numbers of American troops. Plasmodium vivax is the only species known to occur naturally in either of these countries, (1, 2, 8).

Within a month after UN troops entered Korea in June of 1950, malaria began to appear. Total reported new malaria in Japan and Korea in US Army personnel from June 1950, to December 1951, is shown in Figure III. Virtually all new cases of malaria appearing in Japan during that period occurred in returnees and evacuees from Korea, and it has been assumed that all cases were contracted in Korea.

Epidemiological Studies on Korean Malaria - In April 1951, in an attempt to collect limited data on malaria and chloroquin suppression, all hospitals in Japan and Korea were directed to forward smears and a brief summary on each case to the 406th Medical General Laboratory (see Medical Zoology Department Report for chloroquin effectiveness). The epidemiology section attempted to determine the relationships between time of exposure in Korea and the appearance of clinically manifest disease. The cases analyzed in this latter study included only:

(1) Those confirmed by blood film as P. vivax.

(2) Those accompanied by the following historical data:

(a) Date of arrival in Korea.

(b) History of previous attacks of malaria, if any.
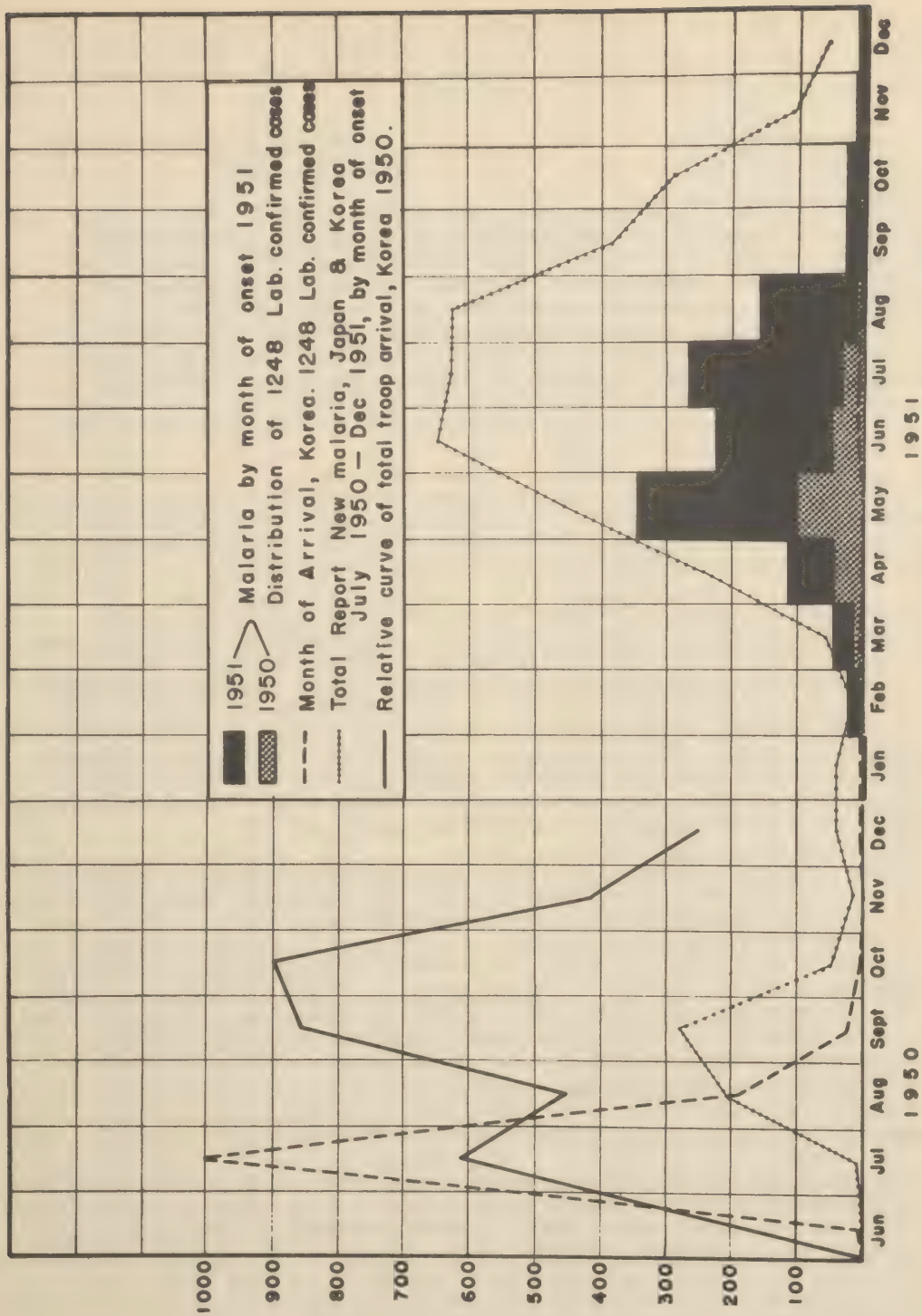
Figure 3. Analysis of 1248 Cases of P. vivax Malaria Contracted in Korea

(c) Date of onset of current attack.

(3) Those with onset of a distinct clinical attack in 1951, who did not experience recurrences throughout the winter of 1950-1951.

(4) Those with no history of malaria between 1 January 1951, and two weeks after their arrival in Korea.

This laboratory has received data on 1248 cases meeting these criteria (Table V and Figure 3). By usual reporting terminology, these cases would all be designated "new" malaria. They are divided arbitrarily into two groups; a) 1950 cases, and b) 1951 cases. The former (1950 cases) had a distinct attack or series of attacks of Korea-contracted malaria in the summer or early fall of 1950 and a distinct fall and winter latent period of at least four months, while the latter (1951 cases) had an initial attack of Korea-contracted malaria in 1951. Only 18 individuals with repeated attacks throughout the fall and winter of 1950-1951 had to be excluded from the present analysis. Two hundred and fifty-nine cases with attacks in 1951 had had prior malaria in Korea during 1950, and were designated 1950 cases. Nine hundred and eighty-nine cases fell into the 1951 group.

The distributions by onset of 1950 and 1951 cases show essentially parallel curves. The peak incidence occurred in May of 1951, considerably earlier than transmission in 1951 would be expected to produce. This would suggest that most clinical malaria appearing in 1951 resulted from infection contracted in the summer of 1950.

Even more striking, however, is the observation that 1006 or 80% of the study cases occurred in individuals who arrived in Korea in June and July of 1950, although an equal or greater number of troops arrived in Korea in August, September, and October of 1950. The relative troop arrivals is shown in Figure 3. Furthermore, of the 1248 cases, only 26 arrived after October 1950, the estimated termination of the 1950 transmission season, although new troops continued to arrive in Korea throughout 1950 and 1951. Although 433 or 44% of all 1951 cases of malaria had their first clinically manifest attack after 1 July 1951, the estimated beginning of the 1951 transmission period, only 23, or 2.3% of these cases arrived after October, 1950. This 2.3% represents the only individuals who of necessity contracted malaria in 1951.

It may be readily seen in Figure 3 that the total laboratory sample represents approximately 30% of the total reported new malaria, and that the sample after August, 1951, is inadequate. This is especially important since September is a crucial period when malaria contracted in 1951 would have been likely to show up. However, the slope of the curve of reported cases is downward from June and does not show a hump in late summer, an irregularity which would be expected if large numbers of 1951 contracted malaria had become manifest. Although not conclusive, these curves when viewed in combination suggest that the great majority of clinically manifest malaria in 1951 was incurred in 1950. By the same token, they suggest that malaria transmission was markedly reduced in 1951, since if chloroquin discipline was such that breakthroughs could occur in the spring and early summer, it is likely that it would have continued to be lax enough to allow newly acquired malaria to become manifest in late summer also.

The average July 1950, arrival actually reached exposure areas in Korea during the third week in July, while the average August arrival reached those areas in mid-August. Hence, July arrivals might be considered chiefly August exposures. The peak of July arrivals in the study group then ties in well with the September 1950, peak of reported clinical malaria. These first clinical attacks in September clearly represent only a small portion of the actual infections delivered, the larger number having been cases with subclinical parasitemia. Assuming that the May-June peak of 1951 is virtually entirely made up of individuals with primary clinical attack of subclinical parasitemia in September, 1950, the latent period was in most cases 8 to 9 months.

The effect of chloroquin suppression on the time relationships shown cannot be defined at present. Chloroquin suppression was offocially initiated in all troops in Korea

Table V. Malaria, P. vivax, Korea, 1951, Showing Month of Arrival and Date of Onset of 1248 Cases Diagnosed by Blood Film at 406th Medical General Laboratory

Month of Arrival in Korea	Jan. 50	Jan. 51*	Feb. 50	Feb. 51	Mar. 50	Mar. 51	Apr. 50	Apr. 51	May 50	May 51	June 50	June 51	July 50	July 51	Aug. 50	Aug. 51	Sep. 50	Sep. 51	Oct. 50	Oct. 51	Nov. 50	Nov. 51	Dec. 50	Dec. 51	Total 50	Total 51	Grand Total
1950																											
June	0	0	0	0	0	0	1	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	4	5
July	0	4	6	11	11	28	39	70	96	217	47	144	27	170	11	97	1	9	3	4	3	3	1	0	245	756	1001
August	0	2	0	5	1	8	0	4	5	20	2	27	4	55	0	34	0	12	1	8	0	0	0	0	13	176	189
September	0	0	0	0	0	0	0	2	0	4	0	3	0	6	0	9	0	1	0	2	0	0	0	0	0	27	27
October	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3	3
November	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
December	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	2
1951																											
January	0		0		0		0			1		0		0		0		0		0		0		0	0	2	2
February			0		0		0			0		0		1		1		0		1		0		0	0	3	3
March					0		0			0		0		0		1		1		0		0		0	0	2	2
April							0			0		0		0		2		0		0		1		0	0	2	2
May										0		0		0		1		0		0		0		0	0	1	1
June												0		0		1		0		3		0		0	0	4	4
July														0		1		2		0		0		1	0	4	4
August																1		1		1		0		0	0	3	3
Total	0	6	6	16	12	36	40	78	101	245	49	175	31	235	11	148	1	26	4	19	3	4	1	1	259	989	
Grand Total	6		22		48		118		346		224		266		159		27		23		7		2				1248

*50 - 1950 cases
51 - 1951 cases

- 17 -

by administrative order in late July, 1950, and was discontinued that year in November. During that period, conditions of supply, distribution, and indoctrination relating to this program were known to be other than satisfactory. However, heavy emphasis was continually placed on malaria discipline and it was considerably improved during the April to November program of 1951. (See Medical Zoology Section Report). It is possible that the peak of malaria incidence in May and June of 1951 represents the survival of only those infections incurred prior to the initiation of chloroquin, thus accounting for the predominance in July arrivals. This would presuppose, however, that infections delivered before chloroquin, but which did not produce a primary clinical attack, had greater survival capacity than infection acquired while a suppressive level is being maintained. In other words, it would suggest a certain prophylactic character beyond and above what would be expected from chloroquin suppressive therapy.

There are several environmental considerations which support the aforementioned impression that transmission was markedly reduced in 1951. Troops in 1950 not only did not have the benefits of mosquito control measures, but, probably more important, they were crowded into the Pusan beachhead of southeastern Korea with throngs of infected refugees. The rural population of southern Korea is known to be heavily infected in several endemic foci (11). In 1951, on the other hand, extensive spraying and also certain natural factors did in fact reduce the mosquito population in Korea markedly. This is also evidenced by the extremely low incidence of Japanese B Encephalitis in Korea in 1951. In addition, troops were no longer concentrated in the Pusan perimeter, but were widely scattered. Front line concentrations were in central Korea in areas which were forcibly cleared of the native population. Troops in rear areas were often living under garrison conditions with vastly greater mosquito protection than in 1950. The actual amount of 1951 transmission will become apparent if troops who leave Korea in 1952 can be adequately followed without curative therapy, and the data on clinically manifest malaria in early 1952 can be collected and correlated.

EPIDEMIC HEMORRHAGIC FEVER: Beginning in October 1951, this section has been engaged in collecting, consolidating and analyzing certain fundamental descriptive epidemiological data on all reported cases of epidemic hemorrhagic fever. In October 1951, as the incidence again appeared to be on the rise in U.N. Forces in Korea, the need for centralized reporting was recognized and a radio reporting system was established by which all cases admitted to hospitals in Japan were reported to this office. Similarly, periodic reports were submitted by the Office of the Surgeon, Eighth U. S. Army In Korea, on cases retained in medical installations in Korea. By this method, name, rank, serial number, race, nationality, dates of arrival in and departure from Korea, onset, admission, disposition and/or death, and unit of assignment on each case became immediately available for tabulation. Subsequently, these patients could be located in Japan for interview regarding specific items of epidemiological interest. The reporting system was made retroactive to include all recognized cases of epidemic hemorrhagic fever which had been diagnosed either at admission or in retrospect since the appearance of the disease in early June, 1951. Detailed analysis of the data assembled in this manner is far from complete. Furthermore, cases are still occurring so that final impressions regarding the current epidemic are not warranted at this time. This report consists therefore of a rough analysis of the epidemiological data collected on cases occurring between 1 June and 31 December 1951.

The first recognized case of epidemic hemorrhagic fever in the Far East Command occurred in Korea in mid-April, 1951. It was an isolated fatal case and was diagnosed in retrospect by review of autopsy material after criteria for pathological diagnosis had been established. Between April and June no cases were reported and careful review of autopsy material revealed no instances of death attributable to this entity. It is probable, however, that non-fatal cases did occur and went unrecognized. Beginning in the first week in June cases ultimately recognized as epidemic hemorrhagic fever appeared in U.N. troops and between 1 June and 31 December 988 cases have been reported. It seems clear thus far that all cases were contracted in Korea, since all patients either developed the disease in Korea or not more than six weeks after their

departure from Korea (See Incubation Period below). No cases have appeared in person-
nel who had never been in Korea or who had arrived in Korea less than two and one-half
weeks prior to onset.

Seasonal Incidence - The distribution of all cases and deaths by week of onset
is shown in Figure 4. The epidemic curve shows two distinct elevations in incidence
without a distinct break. If the curve is arbitrarily divided into two periods with a
break at 8 August, the midpoint of the period of lowest incidence between peaks, cer-
tain rough comparisons can be made. The first period, beginning in the first week in
June with a peak in the last week in July and ending 8 August, comprises 93 cases with
16 deaths, with a case fatality rate of 17.4%. This includes the one isolated fatal
case in April. The second period began 9 August with a peak in mid-November and con-
tinued through the present writing. It consisted up to 31 December of 895 cases with
75 deaths with a case fatality rate of 8.3%. It seems doubtful that the case fatality
actually varied to this degree from season to season, but rather it seems likely that
this was a reflection of poorer recognition of milder cases during the summer when the
disease was new and unfamiliar. As the earlier and milder clinical manifestations
became more widely known, many more cases were recognized and reported in the fall
peak. Nevertheless, on the basis of total number of deaths and the duration of each
wave, it is safe to assume that the autumnal peak was of much greater magnitude than
the one in mid-summer. The seasonal distribution in rates per 1000 per year within
individual major units is shown in Figure 5. It is readily apparent that although
the attack rates varied greatly, each division experienced its highest incidence in
November. These units represent the six American army divisions distributed along the
main line of resistance on the Korean front. Corps and army units and two other div-
isional units are not shown but they likewise showed the same November peak. This
seems to indicate that the disease reached its highest incidence simultaneously along
all parts of the front lines irrespective of geographic location (see below). De-
tailed analysis of seasonal distribution in sub-units are in progress and they reveal
minor variations from unit to unit; however, the general impression of simultaneous
rise and fall of incidence holds true. It will be noted that Division A which showed
by far the highest incidence in Autumn and one of the two highest in July, experienced
no cases at all in June, whereas the divisions with overall low incidences produced
the few June cases. A breakdown of Division A by regiments is shown in Figure 6. All
regiments again show essentially parallel curves, but Regiment 1, which shows the
highest November peak, suffered a relatively low incidence in July. On the other hand,
Regiment 2, whose rate was more than twice as great as that of Regiment 1 in July, suf-
fered a much lower rate than Regiment 1 in November. Regiment 3 had relatively low
rates in both periods.

Geographic Distribution - Accurate knowledge of the geographical distribution
demands precise knowledge of the area of exposure. Since incubation period is not yet
known and at any rate will no doubt prove to have a wide range, it is indeed impossible
in most cases to determine the exact place of exposure. This is all the more difficult
since in a combat zone nearly all patients will give a history of considerable travel
during the possible period of exposure. However, the locations of the major units in
which cases occurred is known, and the endemic area can thus be roughly outlined. Cases
have arisen chiefly in a broad band about 40 - 50 miles deep extending across the center
of the Korean peninsula from Uijongbu and Sangnyong on the west to Inje and Norumegi on
the east. This band is bounded on the north by the main line of resistance and contains
the primary forward troop concentrations. A few scattered cases have occurred in the
central sector as far south as Wonju and one typical clinical case appeared in a soldier
who reported he had never left the Pusan area in south-east Korea. In view of the fact
that considerable numbers of U.N. troops are stationed throughout most parts of south
Korea, this virtual absence of cases south of the Wonju area seems of major significance
in roughly localizing the endemic area. The graphs of incidence by divisions shown in
Figure 5 show the striking difference in attack rate in various sectors of the front.
All these units lie within the broad "endemic band" mentioned above. Beginning with
Division A these units are arranged roughly from west to east along the main line of
resistance. It is apparent that the highest attack rate occurred in the western sector.
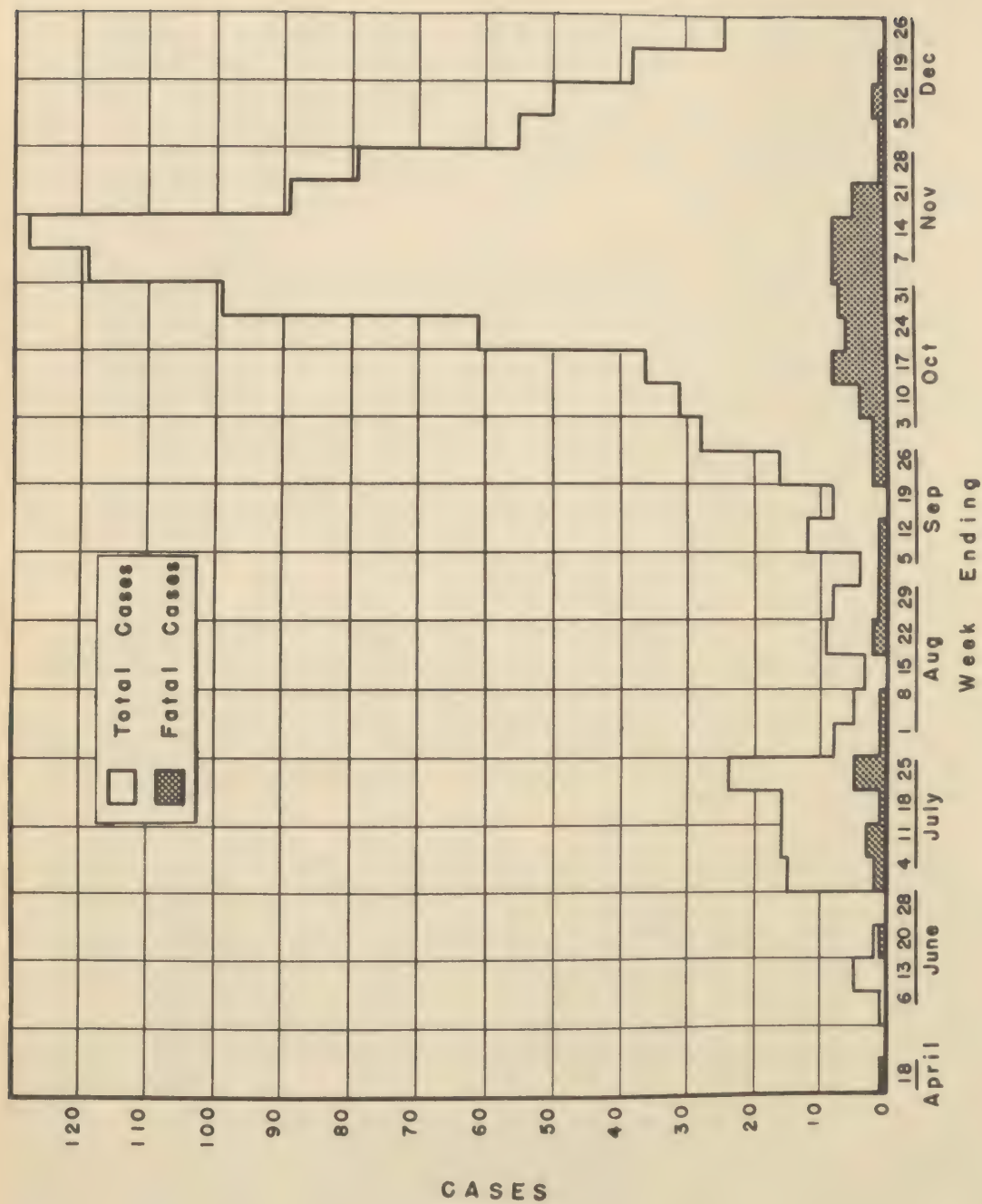
Figure 4. Incidence of Epidemic Hemorrhagic Fever in UN Troops in Korea, 1951, by Week of Onset
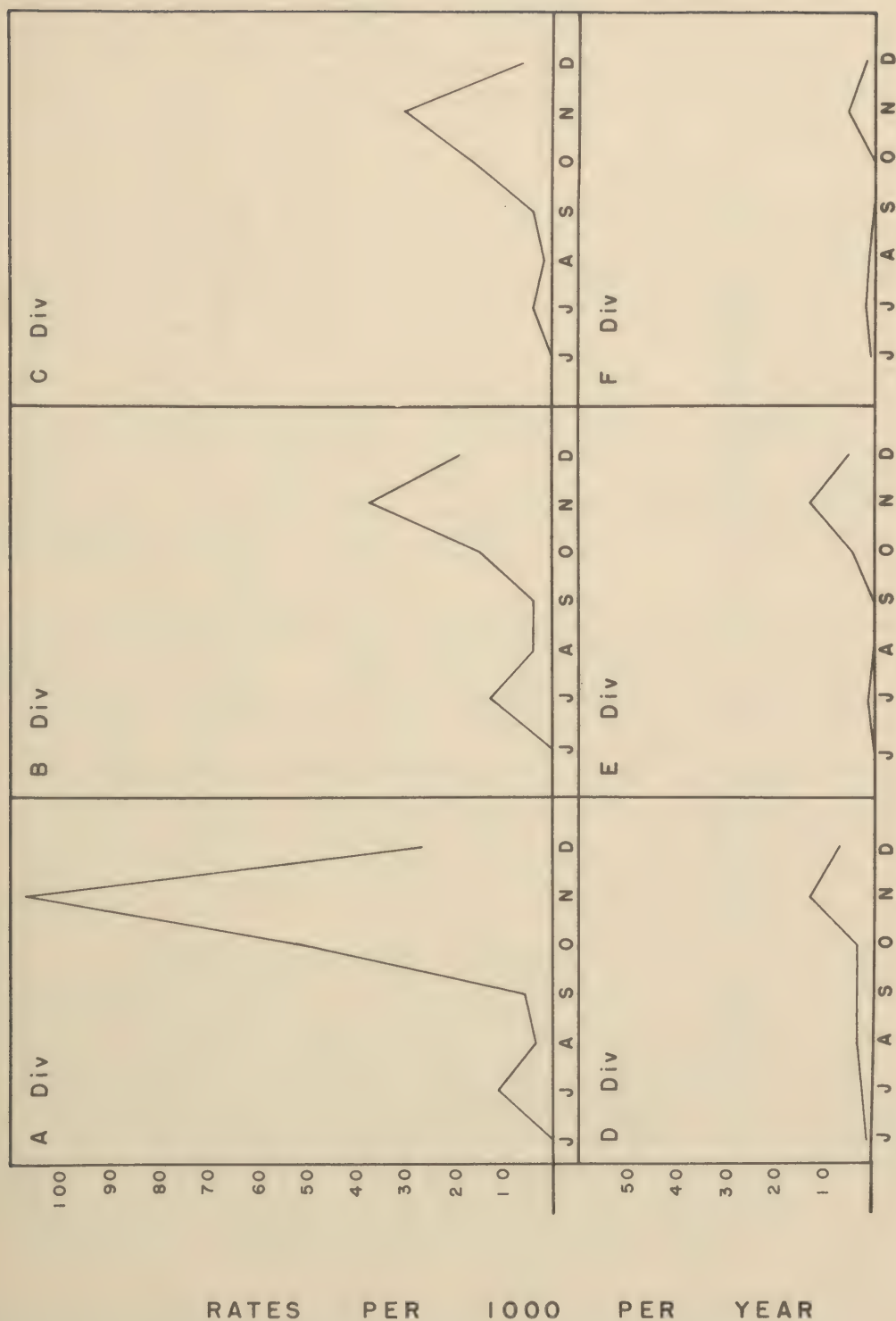
Figure 5. Epidemic Hemorrhagic Fever. Monthly Rates Per 1000 per Year in Six Divisions in Korea

RATES   PER   1000   PER   YEAR
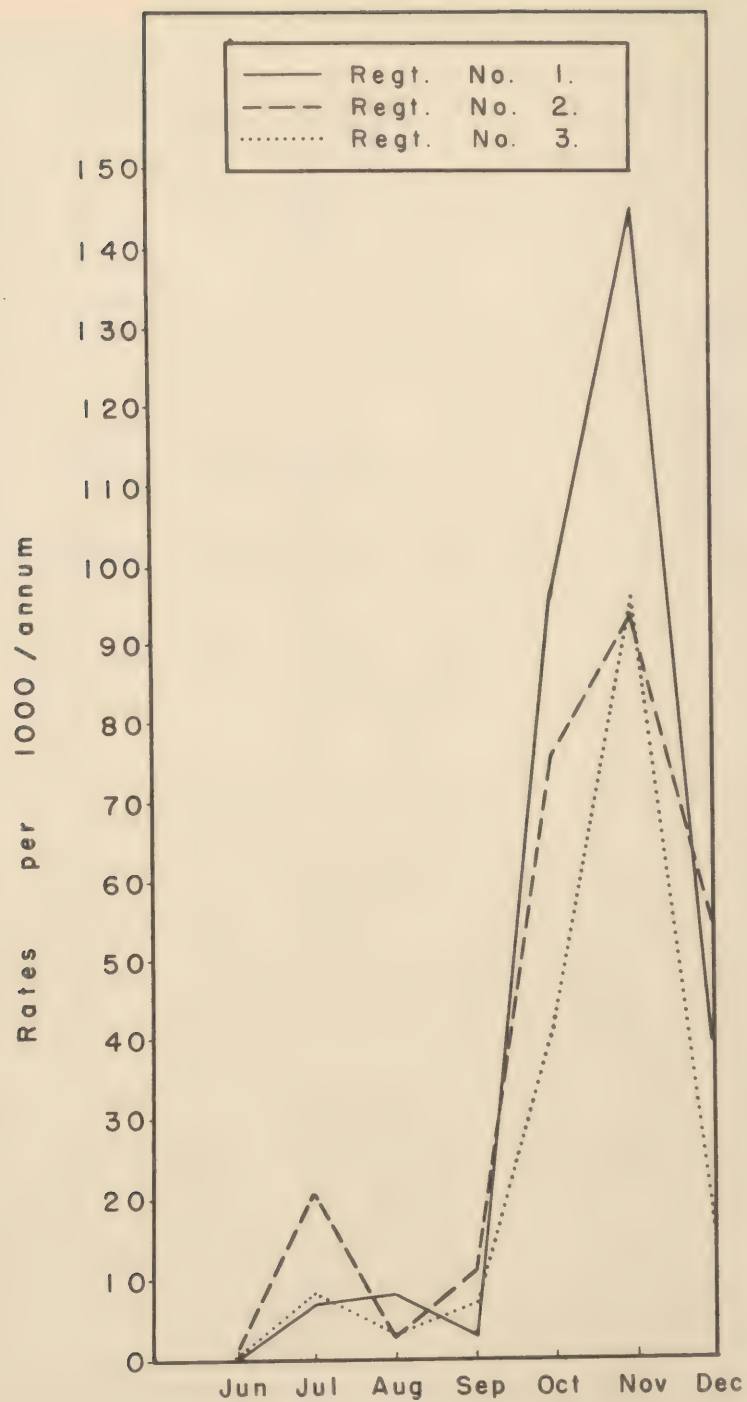
Figure 6. Epidemic Hemorrhagic Fever. Monthly Rates Per 1000 Per Year
in 3 Regiments of Division A

Divisions A, B, and C are situated roughly in the area bounded by Sangnyong, Ch'orwon, Kumhwa, Yongp'yong and Majon-ni. Divisions D, E, and F extend eastward from Kumhwa. One unit of approximately division size is situated directly to the west and south of Division A. This unit is not shown in Figure 5 but its attack rate was quite low, roughly equal to that of Division D. An appreciable number of cases occurred in Eighth Army Units, but these are of little value in helping to describe the geographic distribution of the disease. Personnel of these units are often distributed and attached for duty to forward divisions and it is suspected that most cases occurring in Eighth Army troops were in such persons. Without individual histories the area of exposure cannot be estimated.

The endemic area extends from the valley of the Imjin River on the west with elevations of less than 100 meters to the Southern Taeback Mountain ranges in the east with elevations up to 1000 meters above sea level. The area of greatest concentration of cases includes chiefly the hills north and east of the Imjin valley with elevations of 100 to 300 meters. Although steep (over 30°) terrain slopes predominate throughout the entire endemic area, slopes of low to intermediate grade (below 30°) are more frequently encountered west of Kumhwa in the area of heaviest case concentration. The entire endemic area consists of open grassland with burned over and cut-over areas on the lower slopes and forests of pine, fir and spruce with some oak, birch and maple on the higher, more inaccessible slopes. A few sizeable cultivated areas of rice and dry field crops occur between Kumhwa and Ch'orwon, but in most parts of the area cultivation is spotty and sparse. Similar geographical features can be found in nearly all parts of Korea. It cannot be stated on the basis of available data that the disease has shown any localization to any set of ecological conditions. This does not preclude the probability that within the general endemic area the source of infection is sharply localized to certain spots which may later be found to have specific ecological characteristics.

Incubation Period - Since the source and mode of transmission of Epidemic Hemorrhagic Fever are unknown the time of exposure cannot be accurately determined in any of the cases. Even if these epidemiological details were known the exact day of exposure could not be known in most cases since patients have usually been living in essentially the same environment for many weeks before onset. Certain areas within Korea have produced no cases. However, since there is no proof that the disease is not endemic to those areas, movement into and out of presumed endemic and non-endemic areas cannot be relied upon for exposure data. The only finally reliable limitation on exposure is the day of actual arrival in or departure from Korea. Refinement can sometimes be made in individual cases where specific data regarding geographic location and environmental conditions surrounding the case are known. There has been no evidence throughout the entire epidemic of person to person transmission. No cases have arisen in hospital personnel in Korea except among those who were themselves in the endemic area. No cases have appeared among hospital personnel in Japan.

Maximum Incubation Period - To estimate the maximum incubation period, all cases who departed from Korea prior to onset have been investigated and the following information recorded: (1) Date of departure from the line or other routine duty position, (2) Date of departure from a reserve area if held in reserve, (3) Date of departure from Korea, and (4) Date of onset of symptoms of epidemic hemorrhagic fever. Information of this type received during 1951 is very scanty; therefore, data received on cases with onsets through 31 January 1952 are included in this report. Out of 56 cases having their onset in Japan, 24 cases are considered to have been removed from sources of infection for more than 20 days prior to onset. Some were isolated cases which were observed throughout the autumn epidemic. Twenty-eight cases appeared in troops of one division after their return to Japan in December 1951. In this group 10 cases had onset over 20 days after departure from Korea. Notes on a few of the clinically confirmed cases showing longer incubation periods are presented below:

(1) Pfc. D. C., a member of a rifle company, arrived in Korea 23 July 1951. He was transferred from duty on the front lines to a reserve area on 15 December 1951 and remained in the reserve area until 20 December when he departed from Korea and became permanently stationed in Northern Japan. His onset of Epidemic Hemorrhagic Fever was on 29 January 1952, 45 days after his departure from the front lines and 40 days after departure from Korea.

(2) Sgt. C. T. arrived in Korea May 1951, and was assigned to an infantry regiment. He was withdrawn from the line to a reserve area on 5 December 1951 and departed from Korea on 6 December 1951. His onset of Epidemic Hemorrhagic Fever was 11 January 1952, 36 days after departure from Korea.

(3) Cpl. J. V., a member of a rifle company, arrived in Korea on 6 June 1951. On 6 October 1951, he sustained a shell fragment wound and was evacuated that day to a MASH Hospital near the front. On 11 October he was transferred to a hospital in Japan. He had his onset of Epidemic Hemorrhagic Fever on 10 November 1951, 35 days after being wounded and 30 days after departure from Korea. Since he has been hospitalized from 6 October 1951, it is probable that his incubation period was at least 35 days.

(4) Pfc. R. S., a member of a rifle company, arrived in Korea 13 June 1951. He was withdrawn from the front line 1 December 1951 and stayed in the reserve area from 13 - 27 December. He departed Korea on 30 December and became permanently stationed in Northern Japan. His onset of Epidemic Hemorrhagic Fever was 26 January 1952, 44 days after departure from the front lines and 27 days after departure from Korea.

(5) Cpl. D. M., a member of a rifle company, arrived in Korea 7 October 1951. He sustained a wound of the foot on 10 November 1951 and was evacuated that day to a hospital in southern Korea. On 14 November 1951, he was transferred to a hospital in Japan. He had his onset of Epidemic Hemorrhagic Fever on 7 December 1951, 27 days after entering medical evacuation channels and 23 days after departure from Korea. Since he had been hospitalized since 10 November, it is probable that his incubation period was at least 27 days.

(6) Pfc. V. J., a member of a rifle company, arrived in Korea 15 August 1951. He was withdrawn from duty on the front lines on 16 December 1951 and remained in a reserve area from 16 - 29 December. He departed from Korea 30 December 1951 and became permanently stationed in northern Japan. On 26 January 1952, 41 days after departure from the front lines and 27 days after departure from Korea, he had his onset of Epidemic Hemorrhagic Fever.

In addition to these cases a number of individuals showed intervals of 3 - 4 weeks between last possible exposure day and onset of symptoms. They are as follows: 25 days, 5 cases; 24 days, 2 cases; 22 days, 3 cases: 21 days, 2 cases. It seems clear that the incubation period is often as long as 4 weeks and may occasionally extend to 5 and $5\frac{1}{2}$ weeks. Numerous instances of at least 3 - $3\frac{1}{2}$ weeks incubation period add validity to the 4 week cases.

Minimum Incubation Period - To determine minimum incubation period all cases who arrived in Korea less than 30 days preceding onset have been investigated and the following data recorded: (1) Date of arrival in Korea, (2) Date of arrival in a reserve area; (3) Date of arrival on line or duty position; and (4) Date of onset of symptoms. Cases which would contribute knowledge of the minimum incubation period have been very few in number. Only 10 cases have had their onset of Epidemic Hemorrhagic Fever within 30 days of arrival in Korea and only one case provides evidence of a significantly shorter incubation period than that shown above under maximum incubation period. Notes on that case are as follows:

Pvt. J. P., a member of a rifle company, arrived in Korea on 29 October 1951. He reached the division area on 2 November and assumed his duty position

in the line. His onset of Epidemic Hemorrhagic Fever was 16 November, 18 days after arrival in Korea and 14 days after reaching a known infected area. In view of the fact that the 4 days between arrival in Korea and arrival at his duty station were spent in transit, it seems unlikely that exposure took place before 2 November, giving a maximum incubation period of 2 weeks.

The minimum incubation period may be expected to be more difficult to determine since troops arriving in Korea probably experience considerable delay in reaching exposure areas and conditions while troops leaving Korea usually depart abruptly from a known infected environment so that the termination of exposure can be quickly determined. This delay after arrival may be great but is in almost all cases incalculable and will remain so until the epidemiology of the disease is known in much greater detail.

In summary, limited evidence derived from these observations indicates that the incubation period may range from 2 to 4 and probably 5 weeks. The minimum of approximately 2 weeks has been seen in only one case. With the presently available data, it is impossible to venture a guess as to the mean incubation period.

Early Clinical Manifestations - A member of this section visited forward units in Korea during October and November 1951 in order to collect material from patients during the first few days of the disease for attempts at isolation of the etiologic agent. This entailed careful clinical observation of over 100 early cases during the first few days of disease and follow-up to determine that initial diagnosis was correct. Sufficient detailed data were collected on 81 subsequently confirmed cases so that certain impressions were gained regarding the early clinical manifestations. These observations are presented in summary below.

Symptoms and Signs - Prodromal symptoms rarely occur; if they are present they consist of lassitude and weakness for a few days prior to onset. The onset is usually abrupt and patients are frequently able to state the exact hour at which they noted the beginning of their illness. The first symptoms are headache, chills or chilly sensation, malaise and rarely abdominal pain. Presenting symptoms in 81 cases are tabulated below:

Presenting Symptoms	Cases	Percentage
Headache and chills or chilliness	38	46.9
Headache alone	19	23.5
Chills alone	15	18.5
Abdominal pain	3	3.7
Other	4	4.9
Unknown	2	2.5
	81	100.0

During the first 4 or 5 days temperature fluctuates from 102 - 106°F, usually with afternoon peaks. Intermittent shaking chills are frequent in more severe cases. This febrile period persists through the first week but in the milder cases lysis begins from the 4th and 5th day on. During the first 2 or 3 days the patient is irritable, restless and apprehensive, usually complaining bitterly of insomnia due to headache, abdominal pain and backache. Stupor during the first week is rare but confusion and recent amnesia are not infrequently present.

Headache is a constant feature of epidemic hemorrhagic fever during most of the febrile period. It is usually localized in the frontal or retrobulbar region and it is severe. Ordinary analgesics and even codeine seldom offer relief. It often shows periods of subsidence followed by exacerbations. Low back pain is as frequent as headache. It usually begins a day or two after onset and is so severe that the patient cannot find comfort in any position. The pain is usually localized

just above the sacro-iliac region, but tenderness which is commonly present is usu-
ally elicited over the costovertebral angles. This tenderness is often so marked
that the slightest pressure over the muscle mass produces great discomfort. Myalgia
and arthralgia are common and are most frequently manifested as calf pain and tender-
ness and hip and knee discomfort. Occasionally thigh tenderness and shoulder pain
are noted. Numbness of both arms and legs are mentioned by some patients.

A dry, hacking cough is common but it is usually troublesome only when it ag-
gravates the headache. Examination of the chest usually shows no abnormalities. Occa-
sionally, rales are heard in the lung bases. There is usually a relative bradycardia;
pulse rates of less than 100 are commonly observed with temperatures of 104-105°F.
The cardiac rhythm and sound qualities are normal. Blood pressure is usually lower
than normal and in more severe cases systolic pressure may fall to 80 or even 60 dur-
ing the first 5 days of disease. In rare instances the blood pressure cannot be re-
corded and a picture of so-called "medical shock" supervenes.

Abdominal pain usually begins on the second or third day although rarely it is
a presenting symptom. It is described as cramp-like; it is usually intermittent but
sometimes constant. Exacerbations of pain are usually associated with nausea and vom-
iting which may become severe and intractable. Nearly all patients who complain of
abdominal pain have diffuse tenderness to palpation, more marked in, but not localized
to the mid-epigastrium. The liver and spleen are rarely palpable. Urinary frequency
occurs in about one-third of the cases during the first 36 hours of illness. Thereafter,
it is uncommon. Rarely dysuria is present during the first 2 days.

Complaints referable to the eyes are frequent and variable. They may consist of
severe retrobulbar headache, pain on motion of the eye balls, or less commonly photo-
phobia. Blurring of vision and diplopia are commonly encountered during the febrile
phase, and there is nearly always some degree of suffusion of the conjunctivae during
the first 3 or 4 days. From the 4th day on, small petechial hemorrhages may appear
along the course of scleral vessels. The face is usually flushed, especially over the
malar eminences, presenting an appearance not unlike windburn or sunburn. However, the
upper palpebral fissure which is not affected by windburn or sunburn is markedly dis-
colored in epidemic hemorrhagic fever. This discoloration of the face and neck has
been referred to as "bronzing", and it is associated in many cases with facial, and par-
ticularly, peri-orbital edema. The combined effect of flush, peri-orbital edema and
conjunctival suffusion presents a striking facial appearance which is characteristic of
this disease and may be seen as early as the second day. The throat during the first
two or three days shows engorgement of the superficial vessels especially over the soft
palate and the uvula. In more severe cases the posterior pharynx and buccal mucosa are
involved. Beginning usually about the 3rd day small petechial hemorrhages commonly ap-
pear over these surfaces much as they appear in the sclerae. Showers of these tiny
hemorrhages often develop overnight or in a matter of hours. In some instances they
may coalesce into blotchy areas which are arranged in a linear fashion along the super-
ficial palatal vessels presenting a tigroid appearance. Skin petechiae when they occur
appear simultaneously or a little later over the trunk, usually in the region of the
pectoral muscles in the anterior axillary line. They are fine, pin-point in size, and
often appear in linear groupings. Rarely, they are sparsely scattered over the arms and
legs. Jaundice has not been noted in a single case. Generalized lymphadenopathy in-
volving the anterior and posterior cervical chains, the axillary and inguinal and rarely
the epitrochlear and popliteal nodes may be present. These nodes are discrete and
not markedly enlarged and tenderness is demonstrated in only some instances.

A tabulation of frequency of common symptoms during the first week in 81 cases of
Epidemic Hemorrhagic Fever is shown below:

	Cases	Frequency in Per Cent
Myalgia	74	91.4
Malaise	73	90.1
Backache	72	88.9
Nausea and vomiting	54	66.7
Abdominal pain	53	65.4
Cough	46	56.8
Urinary frequency	25	30.9

Clinical Laboratory Findings - The red blood cell count is normal or slightly above normal with a corresponding elevation of hemoglobin during the first 3 or 4 days. Leukopenia of 4,000-6,000 white blood cells per cu. mm. is the rule during this period. After the 3rd or 4th day of disease the total white blood cell count may rise rapidly to 20,000 or above. Uncommonly, counts of 70,000 and higher have been seen by the 5th day. This rise occurs predominantly in the myelocytic series. However, monocytes may also rise to 12-16% of the total count sometimes suggesting a diagnosis of infectious mononucleosis. Eosinophilia to a slight degree occurs uncommonly. Albuminuria occurs at some time during the disease in nearly every case. It is probably the most common objective feature and should be held as a key point in differential diagnosis. During the first 48 hours, it may be absent or it may appear transiently and to a mild degree. In most cases albuminuria of 2∤ or more will appear on about the 3rd day of illness and by the 5th day 90% of the patients will show this finding. The appearance of large amounts of albumin in the urine characteristically appears rather suddenly. Specimens collected 3 to 4 hours apart often show a rise from a trace to 3∤ to 4∤. Specific gravity usually shows a normal range during the first few days. Microscopically, the urine shows little at first. Usually, only 2 to 3 white blood cells and a similar number of red blood cells per high power field are seen in the centrifuged sediment. Hyaline and finely granular casts are usually present by the 3rd or 4th day of illness.

Discussion - It should be emphasized that most patients with epidemic hemorrhagic fever do not demonstrate the severe hemorrhagic diathesis with which this disease is so familarly associated. The spectrum of clinical severity seen during an epidemic period is remarkably broad and in many cases the only clinical manifestation of vascular disturbance are those described above, which occur commonly in the first week of disease. Fatalities usually occur early in the second week but in those cases in which death occurs during the first week there is a rapidly developing picture of shock with most of the severe clinical manifestations previously described. As far as can be determined it is not possible to predict on the basis of early clinical observations which cases will develop frank hemorrhagic complications. Some cases which appear relatively mild during the early phase have subsequently developed severe hemorrhagic manifestations and have died, while some with a severe acute febrile phase recovered uneventfully after an illness of a little over one week. Although this marked variation in clinical severity presents some difficulty in diagnosis this disease can be recognized with reasonable certainty, in a known endemic area within 24 to 72 hours after onset. By emphasizing early diagnosis, mild cases can be recognized and a much more accurate picture of the incidence of infection within an area can be achieved.

In summary, a patient with a sudden onset characterized by chills, high fever, frontal and retrobulbar headache, low back pain, abdominal pain, nausea and vomiting should suggest a diagnosis of epidemic hemorrhagic fever in a known endemic area. Edema of the face and eyes, bronzing of the skin of the face and neck and relative bradycardia should strengthen this suspicion. If these clinical findings are associated with albuminuria and a leukopenia which later shifts rapidly to a moderate or marked leukocytosis a presumptive diagnosis can be made. Petechiae in the sclerae, mucous membranes and skin do not always appear but when they do the diagnosis can be made with reasonable certainty. Frank gross hemorrhages into the sclerae, the

mucosa of the gastro-intestinal tract and the urinary tract are not common features of this disease during the early febrile phase and may be looked upon as inconstant complications. There is some evidence to indicate that clinical manifestations have been of such short duration in some instances that patients are not hospitalized before subsidence of the diagnostic symptoms and signs.

MEDICAL ZOOLOGY

Current functions of the Department of Medical Zoology include routine identifications of intestinal helminths and protozoa (largely from the Tokyo area), testing of urines for the presence of gonadotropic hormones in suspected cases of pregnancy and neoplasms, checking for malaria among blood donors to the 406th Blood Bank who were previously stationed in Korea, and special or confirmatory identifications of various special parasitologic and zoologic specimens.

Research endeavors include investigations on malaria among troops of the United Nations in Korea; control, epidemiology, immunity, and biology of schistosomiasis and its intermediate snail hosts; and a determination of the extent to which parasitic infections are being contracted by Americans in the Far East. Certain phases of the research program have been in collaboration with parasitologists of the National Institute of Health of Japan, the Kitasato Institute, Tokyo, and the Yamanashi Medical Research Institute.

Other aspects of departmental functions are concerned with the training of laboratory officers and enlisted men assigned to various Army medical units of the Far East Command, supplying type specimens of parasitic forms to these units, and furnishing such specimens, in quantity, to the Distributing Center for Parasitological Specimens at the Army Medical Service Graduate School in Washington, for redistribution to medical schools throughout the United States.

SUMMARY OF ROUTINE EXAMINATIONS: The number of individuals and specimens examined during the year are listed in Table I under the headings of Routine, and Special Projects.

Table I. Summary of Specimens Received

Type of Specimen	No. Individuals Examined	No. Specimens Examined
ROUTINE EXAMINATIONS		
Stool Specimens:		
American	3242	4289
Japanese (Dependents of Americans)	562	562
Japanese	513	521
Malaria Slides from Blood Bank	2007	2007
Urine Specimens (Pregnancy and Neoplasms)	748	800
Special Identifications	120	120
TOTAL	7192	8299
SPECIAL PROJECTS		
Stool Specimens:		
American	2018	3200
Japanese	1656	1974
Malaria Slides (Hospitals and Dispensaries)	2756	3278
Bird Malaria Slides	2500	2500
Sera for Serological Studies (Schistosomiasis and Paragonimiasis)	330	330
Scotch Tape Perianal Swabs for Pinworm	793	793
Blood Specimens for Filariasis	171	171
TOTAL	10,224	12,246

STOOL EXAMINATIONS: During 1951 a total of 5372 fecal specimens were examined from 4317 persons. Of the latter, 3242 were Americans, 562 were Japanese dependents of Americans and 513 were domestic employees of the U.S. Army (Table II). Some interesting comparisons may be made among the three groups. Of these, Japanese employed by the Army showed the highest percent of infection, with the dependent Japanese and the Americans following in that order. For example, 51% of non-dependent Japanese harbored Ascaris, while 32% of the dependent Japanese and 7% of the Americans were infected. The incidence of hookworm for the domestic employees was 20%; this figure fell sharply to 6% among dependent Japanese and to approximately 2% among Americans. In accord with previous observations, Strongyloides stercoralis occurred more frequently in Americans than Japanese. Cases of Taenia rarely occur in the latter.

Such parasites as Ascaris, whipworm, hookworm, and certain others are not a matter of concern among domestic employees as these parasites are not directly transmissible, requiring a period of incubation in the soil. On the other hand, Endamoeba histolytica, pinworm, and the dwarf tapeworm (Hymenolepis nana) can be directly transmitted, although the latter is not commonly encountered among the Japanese. Pinworm is not readily recovered by stool examination, so that the low figure given is not an indication of the true infection rate. Endamoeba histolytica, in general, was no more prevalent in Japanese than in Americans.

Table II. Summary of Routine Stool Examinations for 1951

| | Americans | | Jap. Nat. Dependents* | | Japanese** | |
	No.	%	No.	%	No.	%
No. Examined	3242		562		513	
No. Parasitized	889	27.7	354	62.9	399	77.0
No. with Helminths	374	11.5	283	50.3	363	71.9
No. with Protozoa	547	16.8	129	22.9	109	21.2
Helminths:						
A. lumbricoides	236	7.2	183	32.5	263	51.2
T. trichiura	80	2.4	104	18.5	115	22.4
Hookworm	58	1.7	32	5.7	104	20.2
Trichostrongylus sp.	25	0.8	73	12.9	54	10.5
S. stercoralis	8	0.2	1	0.2	0	0.0
E. vermicularis	12	0.4	0	0.0	2	0.4
Taenia sp.	1		0		0	
H. nana	3		0		0	
C. sinensis	2		2		1	
M. yokogawai	2		0		1	
S. mansoni	2		0		0	
Protozoa:						
E. histolytica	127	3.9	17	3.0	14	2.7
E. coli	217	6.6	58	10.3	72	14.0
E. nana	284	8.7	72	12.8	45	8.8
I. butschlii	22	0.7	1	0.2	4	0.8
G. lamblia	96	3.0	18	3.2	15	2.9
C. mesnili	2		0		1	0.2

* Japanese dependents of Americans

** Domestic employees of U.S. Army

MALARIA SMEARS FROM BLOOD BANK: Beginning in the month of September all donors to the 406th Blood Bank who had served in Korea were examined for malaria. A total of 2,007 individuals were examined. With the exception of one case, all were reported negative; the single positive (Plasmodium vivax) being from a Korean native. On the basis of these findings such examinations have been regarded as unnecessary.

URINE SPECIMENS (PREGNANCY AND NEOPLASMS): A total of 784 urine specimens were submitted for pregnancy determinations; of these, 55 were unsuitable for testing. Six hundred and sixty were tested on Rana nigromaculata male frogs, four on Xenopus laevis female frogs, and in 65 cases both species were used in parallel tests. The small comparative series was the termination of a study on the relative efficacy of the two amphibians. This investigation was reported in the 1950 Annual Historical Report of the 406th Medical General Laboratory. The findings warranted limiting the test to the more available, less expensive, R. nigromaculata. Two specimens of this species are now used for each specimen submitted.

Sixteen urine specimens from male patients with suspected neoplasms were tested for gonadotropic hormones; none of these specimens were positive.

SPECIAL IDENTIFICATIONS: A variety of zoologic as well as parasitic specimens, which required or warranted special consideration, were received from various parts of the FEC. Included among these were definitive parasites, either in toto or in histopathologic sections; fecal samples for identification or confirmation of helminth ova and protozoan cysts; and leeches, snakes and snails from Korea for identification of species. Findings for the 100 specimens received are given in Table III.

MALARIA AMONG UN TROOPS IN KOREA: During 1951 further studies on malaria among troops in Korea were undertaken. Because of the frequency of seasonal relapses among returnees (1), this problem has been of prime interest. Unfortunately, it has been difficult to delineate the exact areas of highest endemicity in Korea, both because of the varied movements of the UN troops and lack of information on location of specific units; consequently, it has been impossible to accumulate confirmatory data for existing reports, such as that of Boyd (2) who recorded minimal occurrence in Seoul, in contrast to severe endemic foci in southern areas. Since the publication of TB Med 208 (3), which listed 1720 deaths from malaria in 1938, no further records have been made available concerning mortality among the Koreans.

Methods - With the continuation of hostilities, this department distributed questionnaires concerning the effectiveness of chloroquine suppressive therapy, to be returned with each slide. In an attempt to discover the actual methods of administration and the degree of discipline enforced, a series of men returning to the United States were selected at random, for interview, from a processing line at Sasebo, Japan; lesser numbers were interrogated in Korea.

Results and Evaluation - Blood smears were received from a total of 2463 persons in hospitals in Japan and Korea, of which 1743 (71%) were positive for malaria (Table IV). All positives were diagnosed Plasmodium vivax, which is in agreement with existing information concerning the prevalence of this species in Korea (4, 5, 6). Although P. malariae has been reported from this country (7), it is to be considered an extremely rare parasite. The same author reported about 20 cases of P. falciparum from Korea but there was no indication that this was a mosquito-borne disease, the majority of the cases being reported from drug addicts who apparently became infected through the use of unsterilized needles. It appears, therefore, that P. vivax is certainly the only malaria of importance, as other species have not occurred in over 1700 positive smears examined.

In general, the identification of malaria parasites by the various hospital laboratories in Japan and Korea have been quite adequate, positive diagnoses being confirmed by this department in most cases, while smears submitted as negative were only occasionally found to include plasmodia.

Table III. Summary of Special Identifications

Type of Specimen	Number	Findings
STOOL SPECIMENS		
Feces from surgical appendices	13	(4) Ascaris; (1) T. trichiura, ova
Feces	31	(4) Ascaris; (2) hookworm; (1) Trichostrongylus sp. (1) H. nana; (3) E.histolytica; (1) E. coli; (2) G. lamblia; (1) T. trichiura; (1) S. mansoni
Feces	2	(2) Ova of M. yokogawai, T. trichiura, hookworm, and Trichostrongylus, sp. confirmed
Feces	1	E. coli confirmed
Peri-anal swab	10	(2) E. vermicularis
ADULT WORMS		
Hookworms	1	Adult of Ancylostoma duodenale
Roundworms	5	Adults of Ascaris
Proglottids	1	Taenia sp.
HISTOPATHOLOGIC SECTIONS		
Lymph node (human)	1	Filarid worm
Appendix	2	(1) Adult E. vermicularis; (1) Adult T. trichiura
Skin nodule	1	Male of Wuchereria sp.
Ulcerated intestine	2	Endamoeba-like bodies (nuclei not clear)
Liver	2	(1) Ova resembling S. japonicum (1) Ascaris ova in embryonation
Lung	1	Ascaris ova in embryonation
PATHOLOGIC SPECIMENS		
Liver cysts	1	Hydatid cysts of Echinococcus granulosus
Liver	1	Adults of C. sinensis
Brain abscess	1	Ova and adult of P. westermani
MISCELLANEOUS SPECIMENS		
Blood Smear	2	(2) Borrelia recurrentis
Urine	1	Only pus cells found; E. histolytica cysts suspected
Tap water	10	Negative; fecal cysts suspected
Leeches from Korea	5	Species: Hirudo nipponia; Herpobdella lineata
Snakes from Korea	2	Species: Elaphe quadrivirgata; E. dione
Snails from Korea	3	Species: Semisulcospira sp.; Hua amurensis

Table IV. Summary of Malaria Smears Received by 406th Medical General
Laboratory During 1951 for Confirmation

Month	Persons Examined	Occurrence of Malaria (P. vivax)	
		No.	%
January	26	6	23
February	308	17	6
March	22	22	100
April	53	47	89
May	190	160	84
June	415	374	90
July	351	312	89
August	422	346	82
September	269	201	75
October	198	160	81
November	153	68	44
December	56	30	54
Totals	2,463	1,743	71

Positive diagnoses of malaria were correlated with questionnaire reports of the adequacy of suppressive therapy. For the latter, three categories were noted: (1) no chloroquine taken, (2) chloroquine taken irregularly, and (3) chloroquine taken regularly. The findings are graphically represented in Figure 1. The cases under consideration were divided into those with no prior history of malaria, and those who had a prior history. The similarity of the two configurations of Figure 1 indicates clearly that both categories constituted a single homogeneous group. Approximately 65% of all persons with malaria had not taken chloroquine, while about 10% had taken it regularly; the remainder had been irregular. These figures were, of course, inadequate without knowing the distribution of the overall troop population relative to the three categories of chloroquine suppression (none, irregular, and regular). In order to furnish this information 439 persons from 264 separate units, most of whom were leaving Korea on rotation, were interrogated regarding the methods of administering chloroquine in their units (Table V). Sixty percent stated that the drug was given by roster; 32% reported that they were required to swallow the drug in the presence of a responsible person, while only 7.5% said that chloroquine was taken on a voluntary basis. Although exact percentages are not ascertainable from these data, it may be concluded that, by far, the majority of men in Korea were receiving, and presumably taking, chloroquine; probably a majority were receiving it regularly. On this basis it is evident that the data in Figure 1 hold considerable significance; 50% or more of the population with adequate therapy contributed only 10% of the malaria cases while the remainder, with either inadequate or no therapy, accounted for approximately 90% of the malaria.

The seasonal occurrence of malaria contracted in Korea was evident from our data; whereas cases were relatively infrequent during the winter months of 1950-51, during which time chloroquine was not given, they began to increase noticeably in the spring. This seasonal occurrence is said to by typical of malaria occurring in temperate zones (1), such as the St. Elizabeth strain (8).

Summary - During 1951 a total of 2463 returnees from Korea were examined for malaria, of which 1743 (71%) were found to be positive for P. vivax, the only species of malaria encountered. The seasonal periodicity of relapses was noted, and observations were made on the effectiveness of chloroquine suppressive therapy. Only 10% of the malaria cases occurred among individuals who had taken chloroquine regularly. Since it has been estimated that at least 50% of the troops took the drug regularly, 90% of the malaria, then, occurred among that half of the troops who took none, or inadequate amounts of the drug.
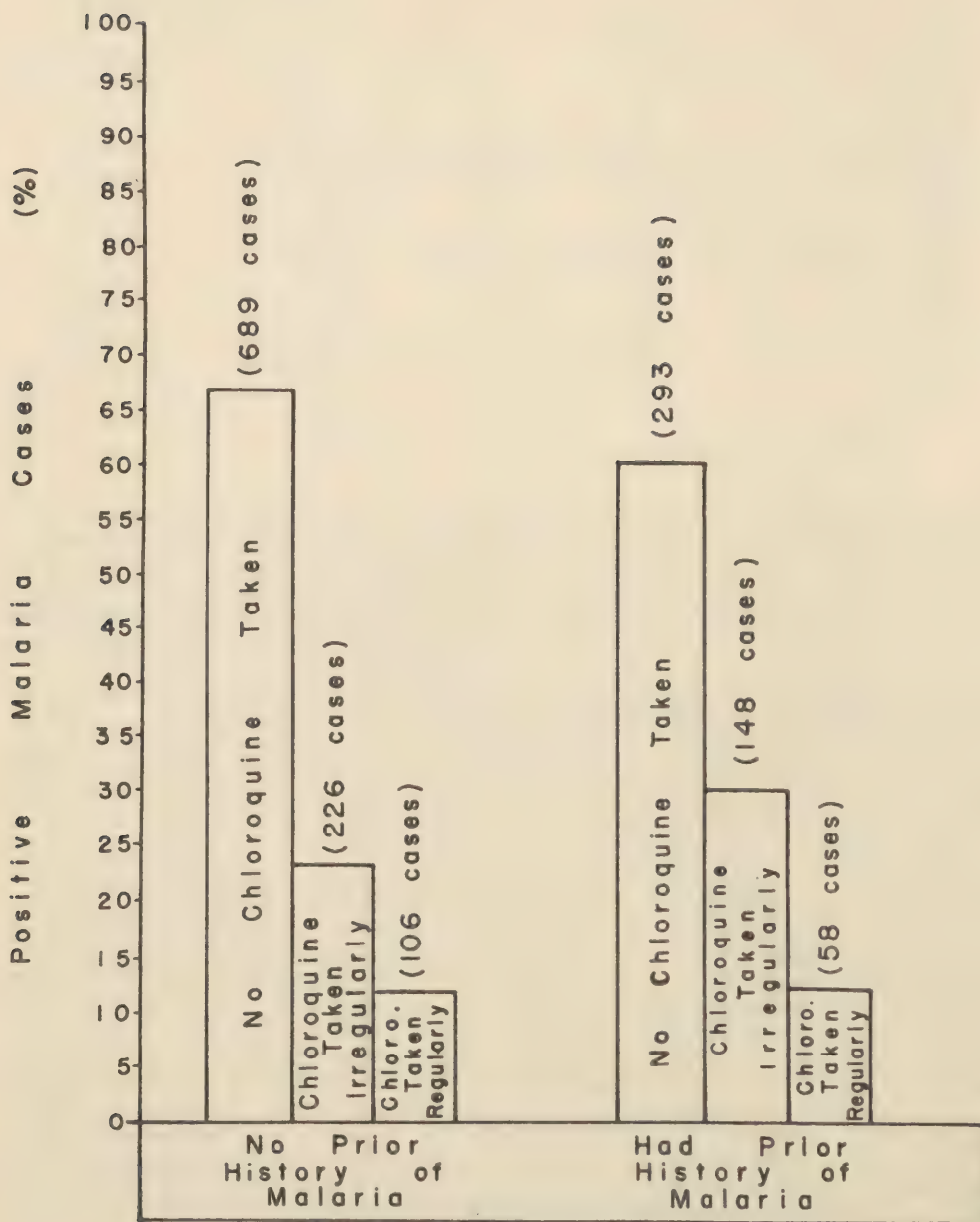
Figure 1. Data on Chloroquine Suppressive Therapy

Table V. Summary of Methods of Administration of Chloroquine in Korea

| Unit | Method of Administration | | | Total No. of Individuals |
	By Roster	Supervised	Voluntary	
Division 1	8	2	1	11
Division 2	27	41	2	70
Division 3	17	3	2	22
Division 4	26	22	3	51
Division 5	15	18	1	34
Division 6	15	16	1	32
Corps A	12	13	0	25
Logistic Command	28	7	1	36
Army Troops	59	8	7	74
Miscellaneous Units	58	11	15	84
Totals	265	141	33	439
	60.4%	32.1%	7.5%	

MALARIA AND OTHER BLOOD PARASITES OF BIRDS: In conjunction with the Department of Virus and Rickettsial Diseases, a series of blood smears from 2100 birds collected throughout Japan have been examined for blood parasites. One or more genera of protozoa, including Plasmodium, Haemoproteous, Leucocytozoon and Trypanosoma, has been recorded for 176, or 8.4% of these birds. Two specimens were found to include microfilariae. These parasites have been found in varying combinations.

SKIN-TESTS FOR PARAGONIMIASIS: A group of 87 persons in Shizuoka Prefecture, Japan, positive for paragonimiasis by sputum and/or stool, were skin-tested intradermally with a merthiolated saline extract of the adult worms of Paragonimus westermani. Results on the first 34 cases of this group were reported previously (9); for the entire number the results are virtually the same as those of the initial group so that only a summary need be given here.

With a 1:10,000 antigen dilution approximately 95% of the paragonimiasis cases demonstrated a wheal which was 3 mm. or more larger than the ones shown by merthiolated saline controls. With the same dilution 30 uninfected persons gave no reaction. When a 1:5000 antigen was used two of the 30 controls were falsely positive. Although the 1:20,000 dilution gave about the same results as the 1:10,000 the latter produced slightly larger wheals and is considered more practicable.

During 1951 a group of Koreans who had been diagnosed as harboring P. westermani were skin-tested with antigens of several parasites, including adult worm extracts (saline) of P. westermani, Schistosoma japonicum, and Clonorchis sinensis. For a total of 136 persons only 87.5% and 83.0% were positive with 1:10,000 and 1:20,000 dilutions of P. westermani antigen, respectively. These percentages are slightly lower than those obtained on Japanese patients. Particularly puzzling was the appearance of false positive reactions with cercarial and adult antigens of S. japonicum as schistosomiasis is not known to occur in Korea; such false reactions had not occurred among Japanese with paragonimiasis. When these same antigens were subsequently tested on known positive cases in Japan good results were obtained. Tabulation of data is presented in Table VI.

Summary - Approximately 95% of 87 known cases of paragonimiasis among the Japanese gave positive reactions to intradermal skin-tests using saline extracts of the adult worm. Of 136 Koreans tested with a similar antigen 87.5% were positive. Curiously, about 50% of the Koreans gave positive reactions when tested with the

Table VI. Skin Tests with Various Kinds of Antigen on Koreans with Paragonimiasis

Antigens (Saline Extracts)	Adult P. wester- mani 1:10,000	Adult P. wester- mani 1:20,000	Adult S. japoni- cum 1:10,000	Cercaria, S. japoni- cum 1:10,000	Liver, O. noso- phora 1:10,000	Adult C. sinen- sis 1:10,000	Saline Control
No. tested	136*	136*	136	136	136	136	136
No. with posi- tive reaction	119	113	69	17	100	76	39
% with positive reaction	87.5	83.1	50.7	12.5	73.5	55.9	28.7

* Of these 136 persons, 94 or 69.1% show eggs in two stool examinations

antigen prepared from adult worms of S. japonicum (a parasite not known to occur in Korea) in contrast to no false positives among the Japanese.

REINFECTION BY ASCARIS LUMBRICOIDES: The ineffectiveness of treatment alone for con-trolling Ascaris and hookworm has been clearly demonstrated (10,11,12,13,14). However, observations relative to this matter have been largely limited to areas where infections were perpetrated by gross pollution of dooryards and adjacent areas.

An attempt has been made to determine how quickly reinfection with Ascaris occurs after treatment in a country where night soil is universally used. A group of teen-age Japanese school children selected following vermifuge therapy were examined monthly for fourteen months. This examination interval allowed observations on seasonal fluctuations in both incidence and density of infections. This project is considered as a basis for further epidemiologic studies.

Results - It will be seen from Table VII and Figure 2 that reinfection or relapse to the extent of 4.6% was manifested by the end of the second month (March) following treatment. The March incidence was quadrupled in April. That of the latter was doubled in May, while in June the figure of 35.6% was no increase over the preceding month. By July the incidence of reinfection had risen to 58.9%; this increase of 23% over the June figure was the greatest recorded for the monthly series. In August the figure was 63.5%, but in September it dropped to 45.0%. From October through January the incidence increased stead-ily to 53.2, 63.4, 65.2, and 80.0%, respectively. Thus the infection returned within twelve months to the original pre-treatment level of 78.2%.

The parasite density also showed changes from month to month which paralleled closely those of incidence (Fig. 3). A marked decrease in the parasite density index, as well as incidence, occurred in September. Whereas the latter increased progressively in subsequent months, there was a delay in increase of the intensity of infection. However, by Jan-uary, 1951, figures for both had reached maximum levels, at which time incidence was essen-tially that of pretreatment level, while the density index was doubled.

Discussion - For the population studied in Yamanashi Prefecture, two seasonal peaks in the severity of Ascaris infection appear to have occurred, one in early summer and another in midwinter. Particularly conspicuous was the decline in frequency and par-asite density of the early fall months, and the marked increase in January. There is ev-idence of a decline in the worm burden during the spring months but the data are not con-clusive.

There can be little doubt that Ascaris infections in Japan result, to a considerable degree, from eating raw or pickled foods which have been contaminated through use of night

Table VII. Data From 14 Months Observations on the Reinfection
By Ascaris lumbricoides

Month after Treatment	No. of Specimens Submitted	Positives No.	%	Parasite Density Index
1 - Feb.	282	0	0.0	0
2 - Mar.	282	13	4.6	39
3 - Apr.	266	44	16.5	45
4 - May	261	99	37.9	172
5 - June	205	73	35.6	124
6 - July	219	129	58.9	182
7 - Aug.	203	129	63.5	130
8 - Sept.	211	95	45.0	45
9 - Oct.	203	108	53.2	103
10 - Nov.	194	123	63.4	103
11 - Dec.	184	120	65.2	88
12 - Jan.	155	124	80.0	321
13 - Feb.	150	95	63.3	288
14 - Mar.	40	26	65.0	132

soil. Explanation of seasonal fluctuations must take into consideration eating habits as well as climate and other conditions.

The pre-patent period of Ascaris in man is about two months (11, 15). The presence of an abundance of embryonated eggs in the environment must therefore antedate peaks of infection by at least two months, which, according to our findings, would be approximately May and November.

According to Ochi (16) almost all Ascaris eggs discharged from the body in winter (November to March) are retarded in development, all reaching the infectious stage in June. Extensive application of night soil, which is practiced throughout late winter and spring, contributes to an accumulation of embryonated eggs in the soil by late spring. The first crops of the year are cabbage, spinach, green onions, radishes, turnips, etc., all of which have been shown to be highly contaminated (as much as 80%) by Ascaris eggs (17, 18). The combination of climatic conditions, plus farming practices and food habits, account for the excessive exposure in May and a peak of patent infections in July.

It has been shown by Takasaki (19) that exposure to direct sunlight (28-40°C) for seven or more hours will kill or deprive an Ascaris egg of its infectiousness. Although Ochi's experiment (16) showed very rapid development in July and August it also showed a higher deterioration rate. In addition to possible destruction of eggs by climatic conditions of the summer, a change in eating habits also occurs. Highly contaminated root vegetables of the spring months are replaced in summer by vegetables less likely to become contaminated, such as tomatoes, eggplant, cucumbers, beans, peas, and corn (18). We have then a possible explanation for the low level of Ascaris infection in early fall. With the coming of fall and more favorable development of eggs, there is a reappearance of leafy and root vegetables in the diet in pickled form as well as fresh. This combination of conditions is offered as an explanation for the increase of infection which leads to a peak in midwinter. It is of considerable import if viable Ascaris eggs do become greatly diminished in the soil at certain times of the year in Japan.

The seasonal fluctuations noted in this study are consistent with the conclusion of Cort et al (20) that Ascaris is very easily voided spontaneously, and that it is necessary to have a constant source of infection to maintain a high incidence. The fact of two or more decreases in the occurrence of Ascaris during the period of one year, as observed in our study, suggests that the life span of this parasite is probably about five or six months.
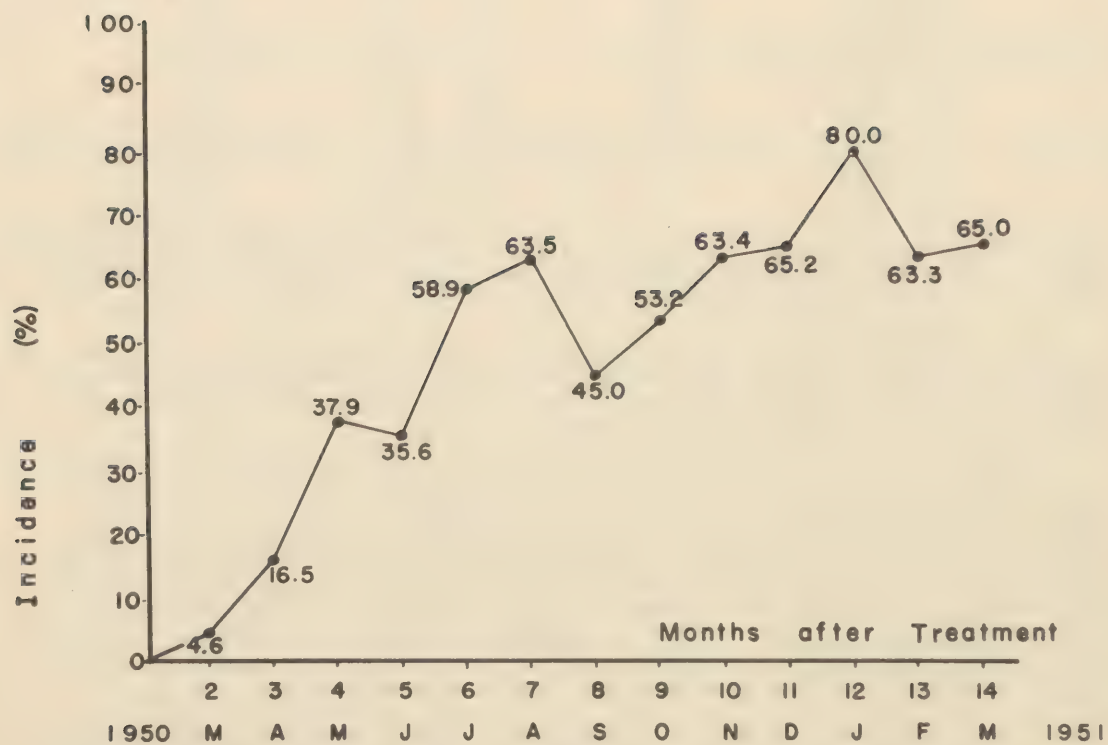
Figure 2. Incidence of Ascaris Reinfections After Treatment
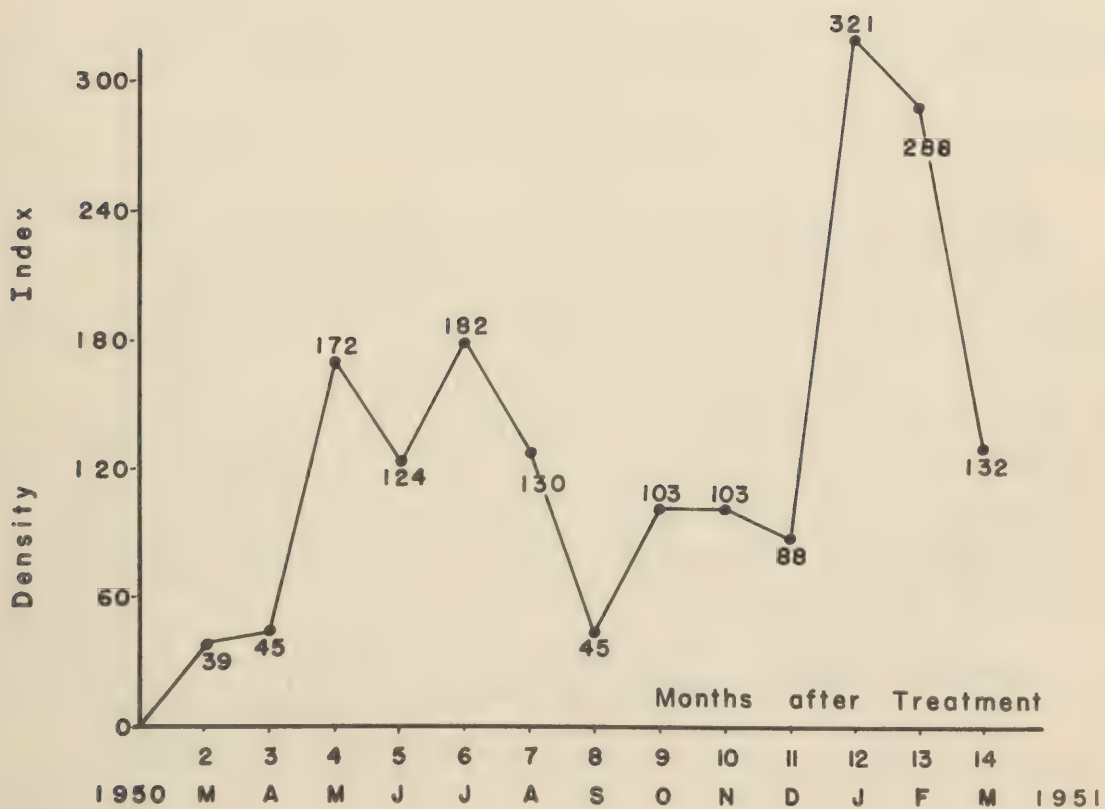
Figure 3. Density Indices of Ascaris Reinfections After Treatment

Summary - Reinfection with Ascaris lumbricoides following treatment has been investigated in Japan where night soil is universally used. Post-treatment monthly examinations were made on a group of teen-age children over a period of fourteen months. Within two months after treatment a few reinfections appeared, increasing to the original level of incidence and intensity after one year. There appeared to be two seasonal peaks, one in midwinter and another during the summer months. A sharp decline in the level of both incidence and density of Ascaris occurred in September due to natural voiding. Possible explanations for these seasonal fluctuations, based on the combined effect of seasonal variations in diet, time of applying nightsoil and effect of climatic conditions, were made.

PARASITISM AMONG OCCUPATION AND COMBAT TROOPS IN THE FAR EAST: Because of the high frequency of many kinds of intestinal parasites in the Orient considerable time and effort was spent, early in the Occupation of Japan, in examining Japanese Nationals who were employed as food-handlers in Army messes. Subsequently, a series of surveys was carried out in various parts of Japan. As a counterpart of this program a project was initiated in 1946 to determine the extent to which Occupation personnel were contracting parasites during their tour of duty in the Far East. Data have been accumulated over a period of five years from numerous Army units, including some in Okinawa and Korea. More recently a group of combat troops in Korea has been examined.

Procedures and Methods - Since it was impractical to examine individuals both at time of arrival and time of departure, the group to be examined was divided, for comparison, into two categories: (1) those who had been in the Far East three months or less and (2) those who had been there longer than three months. In 1946 and 1947 some troops passing through a replacement depot in Japan were examined; many of these had been in combat throughout the Pacific areas. From time to time data have been added for various Army units, including the 8225th WAC Battalion, a number of units of the 1st Cavalry Division, 76th AAA and the 71st Signal Battalion, all of which were located in or adjacent to Tokyo. In 1948 and 1949 Occupation troops in Korea and Okinawa, respectively, were examined. In 1950 and 1951 a total of 789 troops on combat duty in Korea were examined; data for 127 persons who spent over three months in Korea have been segregated. In recent months an opportunity has been afforded to examine troops arriving in Japan directly from the United States; to date, 624 persons have been examined. Also included in the data are findings for over 10,000 patients seeking medical attention at Army dispensaries in and about Tokyo from 1947 to 1950.

Results and Evaluation - For tabulation of data see Table VIII. The control group, as a baseline of comparison, includes 361 Americans who had been in Japan, Okinawa, or Korea (Occupation duty) for less than three months, and 624 who were examined immediately on arrival in Japan. The findings for these groups are similar and have been totalled. For the entire control group of 985 persons, 26.2% were found to be parasitized; 3.7% had helminths and 24.2% protozoa. Hookworm was the most commonly encountered helminth but its incidence was only 1.6%. There were more cases of whipworm than Ascaris. Endamoeba histolytica was found in 4.3%; the figures for E. coli, Endolimax nana and Giardia lamblia, respectively, were 13.5%, 10.4% and 3.4%. Protozoan figures are, of course, based only on cyst recovery.

For the 10,347 dispensary patients, helminth infections (particularly Ascaris) were higher than the controls; the protozoan infections were a little less frequently encountered, though not significantly so.

It should be pointed out that the data on patients are an unrefined consolidation from monthly reports made during the period 1947-1950. No attempt was made to segregate the few cases of Foreign Nationals and Japanese dependents of Americans who were authorized the services of Army medical facilities. Their inclusion accounted, in part, for the high helminth incidence. Presumably, some persons

submitting stool specimens had gastro-intestinal disturbances, yet the frequency of E. histolytica and other intestinal protozoa was not in excess of controls; this may be partly explained by the fact that children, in whom protozoa are less common, were included only among the group comprised of dispensary patients.

Among American troops who had spent more than three months in the Far East, the overall incidence of parasitism was a little higher than that of new arrivals, yet for specific parasites the increases were not statistically significant; furthermore, helminth infections, when acquired, were invariably light. In general it may be concluded that under living conditions of the Occupation, acquisition of parasitic infections in Japan, Korea, and Okinawa has been minimal. The epidemic of amebiasis reported for the Mantetsu apartment building in 1947 (21) constitutes a rare exception to this conclusion and involved relatively few persons.

A group of 426 members of the 8225th WAC Battalion in Tokyo were examined in 1947. All species of helminths occurred with percentages of incidence less than 1%. The figures for E. histolytica, E. coli and E. nana were 4.6%, 11.5%, and 12.9%, respectively.

An attempt has been made to determine the extent to which American forces have acquired parasites in Korea. It has not been possible to examine a sufficient number of persons to allow for other than tentative conclusions; furthermore, time spent in Korea had, in a majority of cases, been of short duration. Data on recent arrivals in the Far East and also on Occupation personnel constitute an excellent baseline of comparison for this group. It is hoped that this investigation of parasitism among combat personnel in Korea may be greatly extended; to date, 789 such persons have been examined and data is available on 127 whose tour of duty exceeded three months.

Marked increases in parasitic infections are not in evidence in our data; in fact, for intestinal protozoa the figures are just slightly lower. For the helminths, however, there is a moderate increase, particularly for the group which had spent over three months in Korea. For these, the helminth figure was 22.8%, in contrast to a figure of less than 5% for new arrivals and Occupation personnel. The increases in incidence of specific helminths, though minimal, appear to be of statistical significance. Whether protozoan infections have not increased, or have actually been suppressed, is not to be decided on data presently available. It might be noted, however, that chloroquine diphosphate, which is used in the field as suppressive therapy for malaria, is also amebicidal. Although the dosage could hardly be expected to eliminate infections of intestinal protozoa, they might have been suppressed to a degree which would reduce the possibility of detecting them.

Summary - During the years 1946-1951 the Department of Medical Zoology examined numerous military units in Japan, Okinawa, and Korea in order to determine the extent to which parasitic infections were being contracted; as a baseline of comparison, 985 persons were examined shortly after arrival in the Far East (3 months or less). More recently, a number of combat personnel in Korea were examined. The incidence of specific helminths for those arriving did not exceed 1.6%. Corresponding figures for persons who had been in the Far East over three months were essentially the same, while for those who had been in Korea for more than three months the incidence for Ascaris, whipworm and hookworm was 11.0%, 10.2%, and 7.1% respectively. The percentages of infection for intestinal protozoa did not show significant differences in the several groups.

"OPERATION SANTOBRITE" - A PROGRAM FOR CONTROL OF SCHISTOSOMIASIS: Beginning in 1945, a concerted study was directed toward the use of molluscacides in the controlling of schistosomiasis. Santobrite (79% sodium pentachlorophenate), DN-1 (dinitro-o-cyclohexylphenol, 40%) and the 20% dicyclohexylamine salt of DN-1 proved very effective against the amphibious snail (Oncomelania nosophora), the intermediate host of Schistosoma japonicum (22,23,24,25). Calcium cyanamide, long used by the Japanese without permanent benefits, has been shown to be quite effective if

Table VIII. Parasitic Infections Among Americans in the Far East

	Americans in Far East 1 - 3 months		Control Groups Americans on arrival in Japan		Totals		Dispensary Patients (1947 - 50)		8225th WAC Bn (1947)	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. Examined	361		624		985		10,347		426	
No. parasitized	99	27.4	159	25.5	258	26.2	3,247	31.4	119	27.9
No. with helminths	17	4.7	19	3.0	36	3.7	1,322	12.8	10	2.3
No. with protozoa	88	24.4	150	24.0	238	24.2	2,362	22.8	113	26.5
A. lumbricoides	3	0.8	1	0.2	4	0.4	820	7.9	3	0.7
T. trichiura	8	2.2	5	0.8	13	1.3	353	3.4	3	0.7
Hookworm	6	1.7	10	1.6	16	1.6	255	2.5	4	0.9
H. nana	0	0.0	1	0.2	1	0.1	8	0.1	0	0.0
S. stercoralis	0	0.0	5	0.8	5	0.5	24	0.2	0	0.0
E. histolytica	14	3.9	28	4.5	42	4.3	395	3.8	17	4.0
E. coli	46	12.7	87	13.9	133	13.5	1,050	10.1	49	11.5
E. nana	40	11.1	62	9.9	102	10.4	995	9.6	55	12.9
I. bütschlii	2	0.6	6	1.0	8	0.8	116	1.1	3	0.7
G. lamblia	11	3.0	22	3.5	33	3.4	373	3.6	9	2.1
C. mesnili	2	0.6	0	0.0	2	0.2	62	0.6	0	0.0

American Occupation Troops in Far East

	in Okinawa (1949)		in Korea (1948) over three months		in Japan 1946-1951		Total Examined		Combat troops in Korea in Korea over 3 months	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. examined	341		241		2,067		789		127	
No. parasitized	111	32.6	73	30.3	708	34.3	223	28.3	42	33.1
No. with helminths	15	4.4	20	8.3	110	5.3	100	12.7	29	22.8
No. with protozoa	102	29.9	62	25.7	647	31.3	149	18.9	20	15.7
A. lumbricoides	2	0.6	5	2.1	27	1.3	51	6.5	14	11.0
T. trichiura	6	1.8	3	1.2	34	1.6	36	4.6	13	10.2
Hookworm	8	2.3	11	4.6	51	2.5	32	4.1	9	7.1
H. nana	0	0.0	0	0.0	1	0.04	0	0.0	0	0.0
S. stercoralis	2	0.6	1	0.4	2	0.1	1	0.1	0	0.0
E. histolytica	16	4.7	12	5.0	96	4.6	40	5.1	9	7.1
B. coli	48	14.1	34	14.1	312	15.1	74	9.4	8	6.3
E. nana	5c	14.7	25	10.4	330	16.0	56	7.1	6	4.7
I. bütschlii	6	1.8	1	0.4	9	0.4	10	1.3	0	0.0
G. lamblia	16	4.7	9	3.7	107	5.2	26	3.3	4	3.1
C. mesnili	0	0.0	0	0.0	1	0.04	0	0.0	0	0.0

properly applied (26). Its use is more expensive than the first two compounds because of the heavy dosage required. Other chemicals, highly molluscacidal, are now known (27), but have not yet been adequately field tested.

On the basis of representative field tests, McMullen (22, 23,24,25) demonstrated the lethal effect of single applications of sodium pentachlorophenate and the above dinitro-compounds. Double applications were made (fall and spring), but in most instances a combination of different chemicals was used; about 1000 feet of ditch were given two applications of sodium pentachlorophenate, but without added benefit. He noted that re-population was in evidence even at the end of the first summer.

The need for determining the advantage of repeated applications of a superior molluscacide seemed to be indicated. The current investigation is a projection of the above work (9), constituting an attempt to demonstrate the possibilities of ultimate eradication through repeated application of sodium pentachlorophenate over a sizeable area. The project was of sufficient magnitude, 150 acres, to serve as a basis for determining whether molluscacides can be made effective and practical for large scale control programs. In addition, data were collected on problems warranting special study.

Results - The results of the four applications of sodium pentachlorophenate are summarily presented in Table IX. The initial pre-treatment collection in the spring of 1950 included a total of 2436 specimens, of which 2364 were alive. The latter figure constitutes a baseline for calculating a percentage of population reduction, an evaluation figure which is used to indicate the status of control before as well as after treatment.

Two weeks after the first treatment (early April 1950) the post-treatment collection of viable snails was only 43, representing a reduction of 98.1% which was supported by findings obtained from control areas.

In October, 1950, 568 viable snails were recovered in the pre-treatment survey, constituting a population reduction of only 76%. The second treatment followed immediately and two weeks later 234 live specimens were recovered, increasing the level of control to 90.1% but giving only a 58.8% reduction for the second application separately, in contrast to approximately 98.1% for the first treatment. There was, however, a delayed or complementary winter kill (in excess of controls) which increased the efficiency of the second application. This was evident in the pre-treatment count in the spring of 1951, when only 32 snails were found, a reduction level of 98.6%.

There remained, then, for the third application only a very low population residue — 32 snails from the 190 quadrats. Its effectiveness on the basis of this small number was disproportionate to that of the previous spring, as 13 live snails were found two weeks following application — a reduction of only 59.4% for the third application; the overall reduction, however, stood at 99.5%.

In the fall of 1951, preceding the fourth application of sodium pentachlorophenate, the pre-treatment count was 115 for the 190 quadrats. This represented an overall snail reduction of 95.1%, which was in contrast to the figure for the preceding fall of 76.0%. Both these figures represented the status of control following a summer period of snail propagation.

The fourth application of the molluscacide accounted for a drop in snail count from 115 to 24 specimens for all quadrats, a cumulative reduction of 99.0%, which is in contrast to a corresponding figure of 90.1% for the preceding fall (second treatment). Assuming a complementary winter kill equal to that of the first winter, the population residue in the Spring of 1952 should be about 0.1%.

Discussion - The choice of chemical for the current project was essentially limited to two: sodium pentachlorophenate and DN-1 (dinitro-o-cyclohexylphenol, 40%). Although the DN-1 compound costs more per pound than Santobrite a smaller dosage is

Table IX. Results of Four Applications Of Santobrite In The
Control of Oncomelania nosophora

| | | Pre-treatment Counts | | | Post-treatment Counts | | | |
Period	No. Quadrats	No. Snails Alive	Dead	Pop. (1) Reduction	No. Snails Alive	Dead	Pop. %(2)	Reduction %(1)
Spring 1950	170	2364	72		43	1300	98.1	98.1
Fall 1950	190	568	34	76.0	234	432	58.8	90.1
Spring 1951	190	32	137	98.6	13	306	59.4	99.5
Fall 1951	190	115	12	95.1	24	65	79.1	99.0

(1) Percentage of population reduction based on orig-
     inal pre-treatment count.

(2) Percentage of population reduction based on indi-
     vidual treatment.

required, so that the actual expense of control is essentially the same for these
two chemicals. The control cost of the dicyclohexylamine salt of dinitro-o-cyclo-
hexylphenol was two to three times that of the others. The availability of Santo-
brite in large quantities arbitrarily made it the molluscacide of choice.* It can
be fairly said that there were no qualities of the sodium pentachlorophenate which
made it a less attractive choice than DN-1. It is soluble in water, did not dam-
age plants and animals of economic importance under the conditions of the experiment,
and its irritating effect on mucous membranes has been overcome by its being man-
ufactured in pellet form.

The choice of Nagatoishi as the center for this investigation proved a desir-
able one. Though near the center of the endemic area where the snail population
was high and widespread, it was well isolated from surrounding areas. The ditches,
which were of two fairly distinct sizes, were largely laid out at right angles to
each other and clearly defined (Figure 4). They were typical of those in areas
where secondary and tertiary (terminal) irrigation ditches constitute the chief
habitat of O. nosophora.

The combined usage of sprinkler and hand pressure sprayers was quite satis-
factory in attaining uniform and thorough coverage of the habitat. A mobile pres-
sure sprayer might have been used in part, but there would have been many places
which could have been reached only by sprinklers and hand sprayers. The latter
are slow but serve well on culverts, holes, etc.

Vegetation constituted somewhat of a problem, which was resolved by cutting.
The possibility of vegetation serving as a protection to snails is a real one, yet
it may also tend to reduce dissipation of the chemical by heavy rains. The effect

* Two tons of the chemical were generously furnished by the Monsanto Chemical
  Co., St. Louis, Missouri

Figure 4. Map of Test Area At Nagatoishi-Cho

of the vegetation has not been clearly demonstrated, except that good results have been obtained even where it was quite dense. Actual scaling or cleaning of surface soil and vegetation was not integrated into the procedure. Whether it would be valuable as well as feasible is not certain.

Because of marked variation in the size of ditches, it has been suggested (23) that a linear-footage standard, which was used in this project for applying a unit quantity of chemical, is an over-simplification; the possible advantage of calculating square footage is evident. However, in the case of our two series of ditches (A - H and I - IX) which were of two distinct sizes, there was little difference in effectiveness. Applications were about equally successful in both, failures being limited to small foci. Apparently, variations in ditch width can be compensated for merely by a rough adjustment in the linear footage to which the unit quantity of chemical is applied.

Considerable difference occurred in the separate effectiveness of the four molluscacidal applications. The reduction in snail population resulting from each was as follows: spring 1950, 98.1%; fall 1950, 58.8%; spring 1951, 59.4%; and fall 1951, 79.1%. The post-treatment percentage of 58.8% for the fall of 1950 was augmented by a winter kill which increased this figure to 94.4%. It appears important to recognize the factors which accounted for such a low fall kill and determine whether a maximum kill can be attained at this time. The third treatment (Spring, 1951) was relatively ineffective (59.4%), which was quite in contrast to the effectiveness of the preceding spring (first application) for which the figure was 98.1%. It may be noted that the collective pre-treatment quadrat count for the third application was only 32 snails. This snail residue was disproportionately of large snails which our data show to be more resistant than small specimens. This may explain the seeming ineffectiveness of the third treatment.

It is reasonable to infer that the population increase during the summers of 1950 and 1951 was due to propagation by survivors. If small snails had occurred among fall pre-treatment collections the point would have been cleared with certainty, but such was not the case. However, this does not rule out propagation as the explanation, since data on growth indicate that June and July-hatched snails may reach a size of 6.5-9.0 mm. by fall (9, 28). To rule out the possibility of snails being carried in by irrigation water, the inlet was screened for several hours on two occasions in 1951; when the pumps were started in the spring, and following a flood period in July. O. nosophora were not recovered on either occasion. The attempt to recover snails from irrigation water seemed advisable as they were abundant in areas adjacent to the inlet canal between the river channel and dyke. Other possible explanations for repopulation include migration of snails from paddies to ditches and overlooking of specimens remaining in hibernation for a prolonged period.

With a post-treatment population level such as existed in the spring of 1950 and 1951, it seemed likely that the reproduction potential would prove to be nil. Approximately one snail per four square feet remained in 1950 and one per 15 quadrats in 1951. Yet in 1950 a count of 43 in the spring increased to 568 in the fall, a 13-fold increase; while in 1951 a count of 13 snails in the spring increased to 115 in the fall, a 9-fold increase. It appears, then, that the propagation potential remains high even when the population level approaches extinction. In explanation, it is known that large snails (8 mm. and over), for which the survival rate was highest, are predominantly female. This fact assumes significance if sperm remain functional in the female for long periods, which is suggested by the fact that copulation is common in the fall as well as in the spring. If copulation should be found to be sharply limited seasonally, it may prove advisable to re-evaluate the time for chemical application.

The results of this investigation indicate that a large scale snail control program for schistosomiasis with sodium pentachlorophenate is practical. A single application of the molluscacide would be inadequate, but an initial and possibly a second

overall coverage, followed by spot treatments for several years where repopulation was in evidence should account for virtual eradication of O. nosophora. At the perimeter, and in many places throughout the endemic area, spot applications might be adequate from the beginning.

Effect of control with sodium pentachlorophenate is almost immediate, with at least a 95% reduction in the snail population within two weeks (spring). Not only is there a reduction in the population, but also a greater number of infected snails succumb. Consequently, the threat of exposure would be markedly reduced, even during the first summer after control is begun. The efficacy of presently available drugs for treatment of the disease is inadequate for mass treatment, so that therapy is advisedly limited to clinical cases. Although existing infections other than clinical ones must largely be ignored at present, the use of sodium pentachlorophenate as suggested above might possibly terminate schistosomiasis as a major public health problem, if a constant control vigil with spot applications of the chemical is continued over a period of years.

Summary - The feasibility of molluscacidal control of the amphibious snail, Oncomelania nosophora, by repeated applications of Santobrite (79% sodium pentachlorophenate) has been demonstrated. This chemical was applied during both spring and fall of 1950 and 1951. The fourth application gave an improved fall kill reducing the population by 99.0%. With a winter kill comparable to that of 1950-51 it is believed that the snails could be reduced to 0.1% of their original density by spring 1952, and that spot applications might result in actual eradication.

Preliminary Field Plot Tests on Potential Molluscacides - Through the cooperation of the Division of Tropical Diseases of the National Institutes of Health, Bethesda, Maryland, and the Dow Chemical Company, Midland, Michigan, 137 organic compounds were made available to us for screening tests. The results of this study were completed and reported in 1950 (9). Nine of the most promising molluscacides were further tested in field plots during 1951.

Methods - Test plots three feet square and with abundant snails were selected on the Chikugo River bottom opposite Kurume, Kyushu. The pre-treatment and two post-treatment counts were made in adjacent one by two foot quadrats. One post-treatment collection was made at the end of three days and the second after 14 days. The snails were carefully collected, measured and tested for viability.

Each compound was tested in the same concentration as that of the Laboratory MLD. In addition, dilutions of one-fifth and one-tenth MLD were also evaluated. A given weight or dosage of chemical/square foot was applied in 100 ml. of water by means of a sprinkling can with a fine nozzle.

Dowcide G, a soluble preparation, was mixed with each of four insoluble chemicals yielding mixtures containing equal dilutions (1:400) of both molluscacides or 1:200 collectively. Tergitol NPG, a non-ionic dispersant, was used to dissolve and disperse the insoluble chemicals. The purpose of these combinations was to determine whether they would have a more rapid and greater lethal effect than the compounds used alone at approximately a 1:200 dilution. Whenever a dispersant was used the ratio to molluscacide ranged from 1:2 to 1:5, depending on the chemical. The use of oil emulsions was avoided at this stage because the molluscacidal effect of the oil would obscure that of the chemical under evaluation. Dosages were corrected for those preparations containing inert substances in order to yield the exact dilution of total active ingredients. Since Dowcide 31 was not dispersable without the use of an oil solvent, the various dosages of this substance were prepared from an emulsifiable concentrate composed of approximately 48% Dowcide 31, 42% xylene and 10% emulsifying agent. To determine the molluscacidal effect of the xylene in the amount used in the 1:250 dilution of Dowcide 31 a special control was used containing all chemicals except the molluscacide itself.

Results and Evaluation - Data on the six best molluscacides found in the field plot tests are summarized in Table X and discussed below the table.

Table X. Chemicals Having the Highest Molluscacidal Effect On
Oncomelania nosophora in Field Plot Tests

Chemical	Dosage: Gms in 100 ml. water/sq. ft. (1)	(2) Dilution	Diluent	Percent of Mortality After 3 Days Exposure	After 14 Days Exposure	Effect of Chemical on Vegetation after 14 Days
Dowcide 2S	1.0	1:100	Water & dispersant	90.3	100	ɛ
Dowcide 2S	0.5	1:200	"	72.9	100	ɛ
Copper pentachlorophenate	0.4	1:250	"	14.5	95	≠
Copper pentachlorophenate	0.2	1:500	"	6.2	95.8	≠
Dowcide G	1.0	1:100	Water	98.1	100	≠
Dowcide G	0.5	1:200	"	71.1	100	≠
Dowcide G	0.1	1:1000	"	80.3	100	≠
Dowcide 31	2.0	1:50	Water, Xylene & emulsifier	25	100	ɛ
Dowcide B	0.05	1:2000	Water	50	46.6	ɛ
2,4,5-Trichlorophenol	0.033	1:3000	Water & dispersant	13.6	19.7	2≠
Dowcide G and 2,4,5-Trichlorophenol	0.5	1:200	"	89.6	100	≠
Dowcide G and Dowcide 2S	0.5	1:200	"	86	100	ɛ
Dowcide G and Copper pentachlorophenate	0.5	1:200	"	18.1	98.4	ɛ

(1) Based on active ingredients.
(2) Dilution of total active ingredients.
  ɛ Slight damage, partially destroyed, may revive.
  ≠ Very slight damage, will recover.
2≠ No effect on vegetation.

Dowcide 2-S (2,4,6-Trichlorophenol 90%) - This is an effective preparation which produced a 100% kill by a dosage of either .5 gm. or 1gm./square foot. When applied at the rate of .1 gm./square foot, only 41.6% of the snails were destroyed. Since Dowcide 2-S is only slightly soluble in water, a concentrated dispersion such as 1:200 may also have a marked residual effect.

Copper pentachlorophenate - A dosage of .2 gm./square foot of this compound was almost as effective as .1 gm. of Dowcide G in an equal area. The compound acts slowly up to three days which is probably due to its insolubility in water. A 1:500 dilution yielded better results than a 1:50 dilution and as good results as a 1:250 dilution. This may be attributed to the more uniform distribution of fine particles of this substance which was obtained in the more dilute dispersion. Strong concentrates of this chemical can be prepared with such dispersants as Tergitol NPG, Sharples 2543, and Antarox B-201. Since copper pentachlorophenate is highly insoluble and dispersed only by emulsifiers, a 1:250 dispersion could be used with less loss by leaching into the soil than in the case of a more soluble molluscacide.

Dowcide B (Sodium pentachlorophenate 75%, sodium salts of other chlorophenols, 13%) - The dosage of this chemical was based on 88% total active ingredients. Dowcide G differs from Santobrite, which has proved to be an effective molluscacide in a large scale test (vide supra) in that it contains 2% less of total active ingredients. It produced 100% mortality in snails when applied in either 1:100, 1:200, or 1:1000 dilutions (1 gm., 0.5 gm., and 0.1 gm./square foot).

Dowcide B (2,4,5-Trichlorophenol, sodium salt 85%) - An MLD at 1:20,000 was secured with this chemical in the laboratory. The respective snail mortalities in the field produced by 1:20,000, 1:4,000 and 1:2,000 dilutions were 13.7%, 14.5% and 46.6% respectively. The similarity of results in the 1:20,000 and 1:4,000 dilutions is inexplicable unless it was due to factors affecting the distribution of the chemical on the soil. This compound merits further testing in stronger concentrations as the mortality from the 1:2,000 dilution was relatively high when compared to the dilutions used for the effective molluscacides.

Dowcide 31 (Chloro-o-phenylphenol 85%) - Xylene and Atlas 1045A in the same proportion as the 1:250 dilution was lethal to 13.7% of the snails. Therefore, some of the molluscacidal effect of the 1:50 dilution was due to xylene since it contained about four times as much as the 1:250 control.

2,4,5-Trichlorophenol - Peculiar results were obtained in these tests since the weaker solutions destroyed more snails than the stronger ones. The MLD of 1:30,000 which had been determined in laboratory tests killed 26.1% of the snails, on contrast to the respective mortalities of 7.6% and 19.7% produced by the 1:6,000 and 1,3000 dilutions. This chemical is easily suspended in water by a dispersing agent and these differences cannot be attributed to lack of uniformity of the dispersions. It seems probable that these irregularities in results may be due to differences in the amount of soil moisture and vegetation in the respective test plots. Factors such as these may interfere with the amount of chemical reaching the snail. In laboratory tests on one molluscacide known to be satisfactory, it was found to be more effective on snails under moist conditions than in dry ones. The operculum and fleshy parts on Oncomelania nosophora are relaxed in the presence of moisture and thus may be initially accessable to the toxicant. After three days exposure, a dosage of .5 gm./square foot, consisting of equal weights of this chemical and Dowcide G, was 18.5% more effective than Dowcide G alone in an equal dosage. This compound is structurally similar to Dowcide 2-S, which yielded almost identical results in combination with Dowcide G. The more rapid lethal effect of 2,4,5-trichlorophenol with Dowcide G may indicate a synergistic action. The lethal effect of single dosages was low, but in view of the high dilutions tested these results may be significant. Further tests appear warranted with this chemical, using stronger concentrations. If the lethal effect increases markedly the result may approach those obtained with other effective molluscacides such as Santobrite.

Relatively unsatisfactory results were secured with the three other chemicals tested: 2-chloro-6-phenylphenol; p-nitro-phenol; and 2,4-dichlorophenol.

Dowcide G in mixture (1:400, each chemical) with either Dowcide 2-S, 2,4-5 trichlorophenol, copper pentachlorophenate or 2 chloro-6-phenylphenol, yielded no improvement over the separate use of a 1:200 dilution of these chemicals; however, the lethal effect of a combination of Dowcide G and Dowcide 2-S was greater after three days exposure than that of each chemical alone, but this advantage was not evident at the end of 14 days. None of the molluscacides damaged the vegetation beyond recovery, although some burned the upper foliage (Table X).

Results of this study indicate that a dosage of chemical in 100 ml. of water gives only a minimum of wetting per square foot, suggesting that improved results might be secured by increasing the diluent without otherwise changing the dosage of molluscacide. This would effect a better distribution of the toxicant, especially in dry areas supporting a dense growth of vegetation.

Summary - Nine potential molluscacides were tested in small field plots but only three, Dowcide 2-S, Dowcide G, and copper pentachlorophenate, were found to be effective. According to observations made 14 days after treatment none of the mixtures of Dowcide G with each of four insoluble chemicals in dispersed form were better than Dowcide G, alone. However, a combination of Dowcide G with either 2,4,5-trichlorophenol or Dowcide 2-S killed snails more rapidly.

Two chemicals, Dowcide B and 2,4,5-trichlorophenol each highly diluted, produced limited but significant results that warrant their retesting in stronger concentrations.

The results from a series of dilutions on each of several compounds indicate that the effectiveness of the chemicals probably was influenced by the nature of the plant growth and amount of soil moisture in the different test plots. These factors must be definitely considered in determining the best dosage of a molluscacide in a practical control program for Oncomelania nosophora.

EMULSIFYING AGENTS SUITABLE FOR DISPERSING SEVERAL INSOLUBLE MOLLUSCACIDES: Emulsification of insoluble molluscacides in oils is not desirable for field evaluation studies, because oils themselves have molluscacidal properties. Consequently, certain emulsifiers were evaluated for dispersing insoluble chemicals. Compatibility tests for combinations of soluble and insoluble molluscacides were also made.

Methods - Twenty-two emulsifying or dispersing agents, mostly non-ionic chemically, were separately evaluated on each of seven insoluble phenol derivatives. One-tenth gram of each molluscacide was added to .5 ml. of each emulsifier and allowed to stand two hours after which it was triturated with a stirring rod and then poured into sufficient water in a mixing cylinder to obtain a dilution of 1:250 or 1:1000, depending upon the compound. Each cylinder was shaken vigorously for 20 seconds and allowed to stand. At 1, 2, 3, 4 and 24 hour intervals observations were made and recorded on the uniformity of the dispersion and the amount of any creamy residue or precipitate formed. If any appreciable sedimentation occurred at these intervals the ease of re-dispersibility was determined by shaking.

A series of compatibility tests for soluble and insoluble molluscacides was made as a preliminary step to determine their effectiveness when used in combination. This procedure was as follows: one-half ml. of Tergitol NPG, containing .1 gm. of an insoluble compound, was added to 99.5 ml. of a 1:1000 (active ingredients) aqueous solution of either sodium pentachlorophenate of sodium trichlorophenate yielding 100 ml., which was a mixture of 1:1000 dilutions of each chemical, or a dilution of 1:500 of total active ingredients. The mixtures were subjected to the same test for uniformity as described for the single dispersions.

Table XI. Summary of Results of Molluscacide Dispersions

Emulsifier and Supplier	Phenol, 2,4,5-trichloro- 1:1000	Dowcide 31 (Phenol, chloro-o-phenyl-) 1:250	Molluscacide and Dilution in Emulsion				
			Phenol, p-tert-butyl- 1:1000	Dowcide 2S (Phenol,2,4,6-tri-chloro-) 1:250	Phenol, 2-chloro-6-phenyl- 1:1000	Phenol Penta-chloro-cu,salt 1:250	Phenol,2,4-dichloro-ro 1:250
Tergitol NPG[1]	A-24	F	A-24	A-24	A-24	p-3	A-24
Polyethylene Glycol 600[1]	A-24	P	A-24	OR-3	CR-4	A-24	OR-1
Polyethylene Glycol 400[1]	A-24	P	A-24	OR-2	A-24	CR-4	CR-1
Skill 234-B[2]	CR-2	P	CR-4	CR-2	p-1	p-1	CR-1
Antarox B-201[3]	A-24	P	A-24	CR-1	A-24	CR-24	CR-1
Antarox A 400[3]	A-24	P	A-24	A-24	CR-1	p-1	CR-1
Antarox A 401[3]	CR-1	P	CR-3	CR-1	CR-1	p-1	CR-1
Antarox A 401/[3] A 402 1:1	CR-1	P	A-24	CR-1	CR-1	p-1	CR-3
Antarox A 401/[3] A 403 1:1	CR-1	P	A-24	CR-1	A-24	p-1	CR-1
Antarox A 401/[3] A 404 1:1	CR-2	P	A-24	CR-1	A-24	p-1	CR-2
Base 401 M [4]	CR-1	P	CR-3	p-2	CR-2	p	OR-1
Trem 615[5]	A-24	P	A-24	p-2	CR-2	p-24	CR-2
Emcol H-77[6]	CR-1	P	A-24	CR-2	CR-1	p-1	CR-1

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Molluscacide and Dilution in Emulsion

Emulsifier and Supplier	Phenol, 2,4,5-tri-chloro- 1:1000	Dowcide 31 (Phenol, chloro-o-phenyl-) 1:250	Phenol, p-tert-butyl- 1:1000	Dowcide 2S (Phenol,2,4,6-tri-chloro-) 1:250	Phenol, 2-chloro-6-phenyl- 1:1000	Phenol Penta-chloro-cu, salt 1:250	Phenol,2,4-dichloro- 1:250
Glycox 1300(7)	CR-4	P	A-24	CR-2	A-24	p-3	CR-4
Emulside 65(8)	A-24	P	CR-4	p-3	p-3	p-1	CR-1
Emulsifier L(9)	CR-2	P	CR-4	CR-3	CR-2	p-1	CR-1
Atlas 1045 A(10)	CR-3	P	A-24	CR-4	CR-3	A-24	CR-4
Atlas 1256(10)	CR-4	P	A-24	CR-4	CR-2	CR-3	CR-1
Triton NE(11)	CR-4	P	CR-4	CR-3	p-1	p-1	CR-1
Sharples 2543(12)	CR-1	P	p-2	CR-3	p-3	A-24	CR-1
Sharples 2481(12)	P	P	p-1	CR-2	p-1	P	CR-1
Sharples 2483(12)	CR	P	p-1	CR-2	p-2	p-1	CR-1

Note: For symbols see Table XII.

Chemical Cos. Cooperating

(1) Carbide and Carbon Chemicals Corp.
(2) Gallowhur Chem. Corp.
(3) Antara Products
(4) E.F. Drew & Co. Inc.
(5) Griffin Chemical Co.
(6) The Emusol Corp.

(7) Glyco Products Co. Inc.
(8) Van Dyk & Co. Inc.
(9) Monsanto Chemical Co.
(10) Atlas Powder Co.
(11) Rohm & Haas Co.
(12) Sharples Chemicals Inc.

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Results and Evaluation - Details obtained on the characteristics of the dispersions are summarized in Table XI. Six out of seven insoluble molluscacides were successfully dispersed in water without the use of an oil solvent. Trials on Dowcide 31 were not successful. Tergitol NPG appeared to be the most versatile dispersant in the proportions used and produced dispersion of each of the compounds except Dowcide 31. Five of these were stable up to the last interval of observation at the end of 24 hours.

Two soluble molluscacides, Dowcide B and Dowcide G, were found to be compatible with four insoluble ones when the latter were dispersed in Tergitol NPG. Although both were compatible, the former (Dowcide B) was considerably superior at dilutions used (Table XII).

The procedure used for testing emulsifiers was a rapid means of recognizing a surfactant suitable for dispersing particular molluscacides; consequently it is possible that some found ineffective might function for other insoluble chemicals. More liberal use of the emulsifier might have increased the efficiency in certain cases. Some of the surfactants were suitable for emulsifying an oil solution of the molluscacide with water, a procedure of practical importance.

In the control of aquatic snails, where toxicants are usually applied to the surface of the water, it seems that an oilless emulsifier-molluscacide mixture, such as we have prepared, may be more effective than one containing oil.

Summary - Twenty-two emulsifiers or surfactants were evaluated against each of seven insoluble molluscacides. Tergitol NPG appeared to be the most versatile for the particular phenol derivatives that were studied. It yielded stable dispersions of each of five different molluscacides. None of the emulsifiers dispersed Dowcide 31 unless the latter were first dissolved in an oil. The compatibility of two water soluble molluscacides in combination with insoluble ones dispersed in Tergitol NPG was demonstrated.

THE INFLUENCE OF VARYING AMOUNTS OF SOIL MOISTURE ON THE EFFECTIVENESS OF SODIUM PENTACHLOROPHENATE AS A MOLLUSCACIDE: It had been observed during "Operation Santobrite" that four degrees of soil moisture occurring normally in the habitats of Oncomelania nosophora could be arbitratily distinguished, namely: areas perfectly dry at intervals during the year; moist areas; thoroughly wet sections; and muddy localities such as occur near the water line of ditches or depressions. The following experiment was performed to determine whether these varying amounts of soil moisture had any bearing on the lethal effect of Santobrite.

Methods - Approximately 30 to 36 viable snails of the same general size were uniformly distributed over the soil in each of four clay vessels (7" in diameter and 3" deep). The soil moisture was varied to simulate field conditions, that of the wettest being soil covered by a layer of water approximately 1/16" in depth. All pots were covered to eliminate evaporation.

Careful calculations were made to assure that the chemical, applied as a fine spray, was of the same concentration as that used in the field. Snail movements were noted and snail viability was determined after 96 hours. Three trials were performed, using snails of a different size.

Results and Evaluation - The summation of the several trials (Table XIII) indicates that the molluscacide became increasingly effective as the soil moisture increased. Progressively, from driest to wettest conditions, the mortality rates were 58.8, 62.8, 70.8, and 80.8%. Although the difference between the two drier soils was minimal, the contrasts between these and the two wetter conditions is certainly significant. It should be pointed out that larger amounts of water accounted for a higher snail kill in spite of the added dilution of the chemical, which was considerable; it should be noted also, however, that a 1:200 concentration of Santobrite was applied.

Table XII. Data on Mixtures Containing Equal Dilutions (1:1000) Of Soluble and Insoluble Molluscacides

Soluble Molluscacide 1:1000 - 99.5 ml	Insoluble Molluscacide, .1 gm in 0.5 ml Tergitol NPG			
	Phenol, 2,4,5-trichloro-	Phenol, 2-chloro-6-phenyl	Phenol, 2,4,6-trichloro- (Dowcide 2-S)	Phenol, penta-chloro-, copper salt
Dowcide B Phenol, 2,4,5-tri-chloro-Na salt 85%	A-24	A-24	A-24	A-24
Dowcide G Phenol, pentachloro-Na salt 75%	A-24	CR-4	CR-4	CR-5

Symbols:

A-24 Uniform dispersion, up to last observation interval, of 24 hours

CR Slight creamy residue, easily redispersed

CR Oily residue, difficult to redisperse, breaks quickly

P Molluscacide precipitates when added to water

p Slight precipitate

Numbers indicate the approximate hour at which the above reactions occur

Table XIII. The Effect of Moisture on the Molluscacidal Action of Santobrite

Soil Condition	Snails 4 - 7 mm			Snails 7 - 7.5 mm			Snails 7.5 - 8.5 mm			Summation		
	No. Used	Dead No.	%	No. Used	Dead No.	%	No. Used	Dead No.	%	No. Used	Dead No.	%
Dry	34	26	76.5	30	20	66.7	33	11	33.3	97	57	58.8
Damp	29	21	72.4	30	21	70.0	35	17	48.6	94	59	62.8
Saturated (no free water)	32	31	96.9	30	27	90.0	34	10	29.4	96	68	70.8
Muddy	31	31	100.0	32	29	90.6	36	20	55.6	99	80	80.8
Total	126	109	86.5	122	97	79.5	138	58	42.0	386	264	68.4
CONTROL												
Dry	30	0	-	38	0	-	33	0	-	101	0	-
Damp	27	1	3.7	30	0	-	35	0	-	92	1	1.1
Saturated (no free water)	22	0	-	29	0	-	36	0	-	87	0	-
Muddy	29	3	10.3	31	0	-	36	0	-	96	3	3.1
Total	108	4	3.7	128	0	-	140	0	-	376	4	1.1

As the size of the snail increased there was a lessening in the mortality rate for all soil conditions; e.g., from smallest to largest snail sizes the figures for the wettest soils were 100%, 90.6%, and 55.6%.

Although the overall mortality rate was considerably less than 100%, it was noted that many of the surviving snails, after only 96 hours, were unable to crawl even after they had been in fresh water for 12 hours. In general, it is believed that improved snail control can be obtained with Santobrite if the chemical is applied shortly after a mild rain while the soil is wet. Possibly, increasing the volume of water in which the chemical is dissolved for dispersal without changing the basic rate of chemical application would also enhance the molluscacidal effect on dry soils.

Summary - Increased moistness of the soil appeared to improve the effectiveness of Santobrite as a molluscacide. Larger snails are evidently more resistant to the lethal effects of the chemical than smaller ones. Increasing the amount of diluent without changing the basic rate of chemical application appears to be a possible means of increasing the molluscacidal effect when the environment is unduly dry.

EFFECT OF SANTOBRITE ON THE EGGS OF O. NOSOPHORA: In April a considerable number of eggs were laid by O. nosophora under laboratory conditions. Some of them were used to determine the destructive effect which Santobrite might have on them. Four to six eggs in their natural fecal encasements (vide infra - section on Egg-laying and Incubation), containing fully developed but unhatched snails, were placed in each of six petri dishes containing soil saturated with water. The egg cases, clinging firmly to small pieces of filter paper or leaves, rested on the surface of the soil, partially submerged in water. The area of soil surface was calculated and a dosage of Santobrite, equivalent to that used in the field, was applied by means of a small florists' syringe. Similarly, other exposures were made with dilutions of 1:1000, 1:4000, 1:8000, and 1:10,000. A sixth dish prepared by the same methods, except unsprayed, served as a control.

At the end of five hours one egg from each dilution, including the control, was removed and dissected to determine viability. All the remaining eggs were tested for viability after 24 hours exposure to the chemical. Eggs in all dilutions of Santobrite were dead at the end of either five or 24 hours. All eggs in the control were alive. Thus it appears that Santobrite is not only lethal against adult snails, but also against their eggs.

THE EFFECT OF LIGHT ON THE SHEDDING OF CERCARIAE OF SCHISTOSOMA JAPONICUM FROM ONCOMELANIA NOSOPHORA: Relatively limited experimental work has been carried out to determine the influence of light on the shedding of the cercariae of Schistosoma japonicum from the intermediate snail host. Variable results obtained may be due to the fact that the several investigations involved the use of different species of Oncomelania. Isobe (29) studied the influence of light in relation to O. formosana; Bauman et al (30) used O. quadrasi, the Philippine snail; Mao et al (31) used O. hupensis, the Chinese snail; and Osaka (32) working in Japan, used O. nosophora.

An initial step has been taken in the clarification of this problem by performing experiments relative to the effect of light on shedding cercariae from O. nosophora. A complete understanding of the problem may require further experimentation on the several snail species, including not only the influence of light, but also the daily shedding pattern and the means by which the cercariae actually leave the body of the snail.

Methods - In each of two experiments approximately 60 snails, from a colony with an incidence of infection of about 50%, were placed separately in water-filled sputum cups and covered with glass slides. Half the snails were placed in a totally darkened cabinet, while the others were left under natural daily changes of light and

darkness to act as controls. Dechlorinated tap water with a pH of approximately
7.0 was used and daily cercarial counts were made at 0800 hours. For the first
experiment these counts were made for a period of four days. In the second they
were made for 11 days, after which the snails under total darkness were exposed
to the natural cycle of light and darkness and counts continued for an additional
three days.

Results and Evaluation - Tabulation and graphic representation of data
are presented in Table XIV and Figure 5. In the first experiment there were 17
infected snails among those placed in darkness, for which there was a cumulative
shedding of 188 cercariae per snail for the four-day period. In contrast, ten
positives among control snails exposed to the natural daily cycle of light and
darkness shed a cumulative average of 949 cercariae.

Table XIV. Effect of Light on Shedding of Cercariae

| | Experiment No. I | | Experiment No. II* | | |
Condition	Number Positive Snails	Cumulative Ave. Cercariae/Snail 0-4 days	Number Positive Snails	Cumulative Ave. Cercariae/Snail 0-11 days	Cumulative Ave. Cercariae/Snail 11-14 days
Light	10	949	16	1113	1181
Dark	17	188	19	385	1192

* Snails in darkness transferred to normal daylight on 11th day.

For the initial phase of the second experiment the results were comparable to
those of the first. Nineteen positive snails in darkness averaged 385 cercariae for
the eleven-day period, while 16 with exposure to light averaged 1113 for the same in-
terval. That light does somehow influence emergence of cercariae from the snail was
made increasingly evident when the snails kept in total darkness were allowed exposure
to light. Whereas the latter had released only one-third as many cercariae as the con-
trols at the end of eleven days of darkness, cercarial counts were almost identical for
experimental and control groups after the additional three days with equality of light.

The findings of the current studies indicate clearly that cercarial shedding of
S. japonicum in O. nosophora is influenced by light. The absence of light, however, is
only partially inhibitory, shedding under total darkness being about one-third that
occurring under the natural cycle of light and darkness. These findings for O. nosophora
are similar to those found for the Chinese species, O. hupensis, by Mao et al (31) who
found darkness to be equally inhibitory at temperatures of 13-14°C, 15-17°C and 25-27 C.
In contrast, Isobe (29) observed that darkness enhanced emergence of cercariae from
O. formosana. Similarly, Bauman et al (30), working with O. quadrasi, noted strong light
to be partially inhibitory. Even though the shedding of cercariae from O. quadrasi is
nocturnal, the last named workers assumed that light and darkness were not the control-
ling factors since cercariae did not emerge under artificial darkness during daylight
hours. More recently Olivier (33) noted a very definite relationship to light in the
case of Schistosomatium douthitti; a reversal in daily shedding time followed almost
immediately when the daily light periodicity was experimentally reversed.

The observations on the relationship of light to emergence of schistosome cercariae
in these four closely related species of snail suggest the existence of a distinct phys-
iological difference among them. It appears likely that cercarial emergence for O. for-
mosana is nocturnal, as has been shown to be the case for O. quadrasi (30) while for
O. hupensis the cercariae probably leave the snail during the daytime as has been demon-
strated for O. nosophora (9, 34). Light, in some manner still completely unknown, is
probably the critical factor in this relationship.

Summary - It has been shown that cercarial emergence from O. nosophora is
greatly suppressed in the absence of light. Infected snails in darkness under otherwise
favorable conditions for shedding gave off approximately one-third as many cercariae as
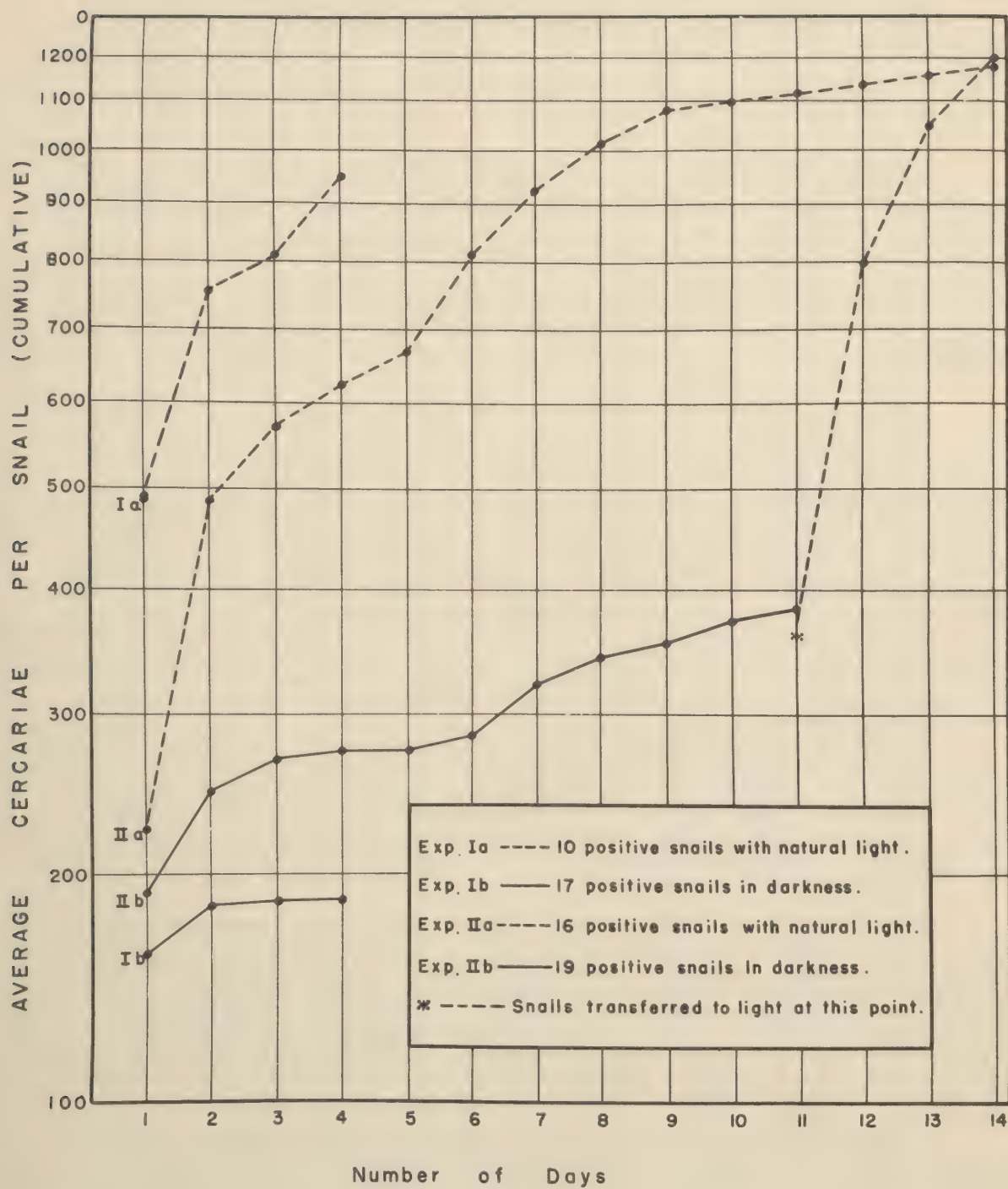
Figure 5. Effect of Light On Shedding of Cercariae of <u>Schistosoma</u>
<u>japonicum</u> from the Snail Host <u>Oncomelania</u> <u>nosophora</u>

controls over a period of 11 days; subsequently, experimental snails were transferred to light and their shedding sharply increased to equal that of the controls.

EFFECTS OF TEMPERATURE ON SHEDDING OF CERCARIAE OF SCHISTOSOMA JAPONICUM FROM ONCOMELANIA NOSOPHORA: Inconsistencies have characterized the results of experiments relative to the effect of temperature on the shedding of the cercariae of Schistosoma japonicum (29, 30, 31, 32). The influence of this factor on cercarial emergence in the case of O. nosophora has recently been investigated. Temperatures used ranged from 6°C to 35°C.

Methods - On each of several occasions, about 60 snails with a high incidence of infection were placed separately in water-filled sputum cups which were covered with glass slides. Equally divided, they were exposed to refrigerator, room and incubator temperatures; in repeated tests the temperatures of refrigerator and incubator were varied. Snails at room temperatures were essentially controls, but in addition, experimental snails were transferred from refrigerator and incubator to room temperatures after the experiment was completed, in order to note potential shedding. Most tests thus far have been carried out in darkness, which imposes a partial suppression of shedding, but allows an adequate experimental range for detecting the influence of temperature.

Results and Evaluation - For tabulation of data see Table XV. No shedding whatsoever occurred at 6°C in darkness; even under the influence of light, suppression was complete at 8°C. That temperature was the suppressing influence is evident by comparison with controls, and also by emergence of cercariae when the experimental snails were transferred to room temperature. Ten degrees (10°C) was the lowest temperature at which shedding occurred, but only half the positive snails shed and the average number of cercariae was very low. At refrigerator temperatures of 12 and 15°C almost normal shedding occurred, but it was somewhat delayed. At the maximum temperature of 35°C only a limited number of cercariae emerged, and these exhibited a shriveled distortion. A considerably larger number were recovered at 30°C but these were also distorted. In the incubator, however, at 28°C and 26°C they were normal in appearance and activity. The numbers of cercariae shed at these temperatures were not markedly greater than those obtained under room conditions at $21-23^{\circ}$C, which in turn were not significantly different from those at 12 and 15°C.

On the basis of these data from small numbers of snails, it appears that cercarial emergence is relatively uniform over a wide range of temperatures ($12-28^{\circ}$C), and that shedding and the resulting threat of exposure may be possible at any time that the snail is active. We have noted that the snail environment goes as high as 30°C during the extreme heat of the day, but the daily peaks of shedding occur in the last half of the afternoon, with considerable shedding in the evening (9, 34) when temperatures are reduced.

The only report regarding the emergence of cercariae from O. nosophora is that of Osaka (32) who noted that it required more time at $18-24^{\circ}$C than at $25-29^{\circ}$C. Isobe (29) reported similar findings for O. formosana and noted that 15°C was the lowest at which cercariae emerged. Bauman et al (30) reported an optimum temperature range of 19-30 C for O. quadrasi, in contrast to our findings of $12-28^{\circ}$C for O. nosophora. This difference is consistent with the fact that O. quadrasi exists in a tropical environment. The optimum range for O. hupensis was noted by Mao et al (31) to be from $15-35^{\circ}$C, with 5°C as the lowest temperature at which shedding occurred. The contrast between their maximum figure, and that of our findings, is considerable.

Summary - Shedding of cercariae of S. japonicum from O. nosophora seemed to occur normally within a range of $12-28^{\circ}$C. Higher temperatures appeared to have a harmful effect on the larvae, which must finally be determined by their infectivity for the definitive host. Eight degrees (8°C) and lower were completely inhibitory. Except for periods of hibernation, it appears that temperature is not a limiting factor for shedding in the case of O. nosophora.

Table XV. Effect of Temperature on Shedding of the Cercariae of S. japonicum

Experiment Number	Experimental Conditions and Results				Additional Shedding under more Favorable Conditions		
	Conditions	Number Snails Shedding	Positives failing to shed	Av. No. Cercariae Shed	Conditions	Number Snails Shedding	Av. No. Cercariae Shedding
1	6°C dark	0	6	0	23°C dark	6	368
5	8°C light	0	4	0	20°C light	4	278
6	10°C dark	6	6	19	20°C light	12	311
3	12°C dark	6	0	487			
2	15°C dark	12	1	507			
Controls at Room Temperature							
1	23°C dark	7	0	209	23°C light	7	1350
2	22°C dark	5	0	1172			
3	21°C dark	7	0	649			
4	21°C dark	5	0	333			
Total		24	0	564			
3	26°C dark	6	0	770			
4	28°C dark	2	0	390			
6	28°C dark	5	0	630	28°C light	5	96
2	30°C dark	5	1	287*			
1	35°C dark	11		59*	23°C dark	8	116

* Cercariae exhibited a shriveled distortion

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OBSERVATIONS ON LAYING AND INCUBATION OF EGGS OF ONCOMELANIA NOSOPHORA: Investigations on laying and incubation of eggs of Oncomelania nosophora were first made by Sugiura (28). Subsequently, Abbott (35) and McMullen (36) reported similar observations on O. quadrasi.

During May 1951, eggs of O. nosophora were incidentally noted in the laboratory. Subsequently they were laid in such numbers that a series of investigations could be made on egg-laying habits of the snail; it was also possible to collect data on the incubation period.

Methods - Egg-laying was observed mainly in petri dishes where the snails maintained on filter paper with decaying leaves and straw as a source of food. This laboratory environment, which was described by McMullen (23, 25, 37) has proved a very effective way to maintain mature snails. Eggs were transferred from laying chambers to other petri dishes at two-day intervals and observed for hatching. Young snails were transferred regularly upon hatching and the numbers recorded at such times.

Observations - Eggs were laid singly, particularly on decomposed leaves and straw included as food, and each egg was separately encased within a dome-shaped covering of snail feces. This observation of fecal encasement of eggs was reported by Abbott (35). Under the conditions of the current experiment there was no doubt that the use of feces as there was no other substance available in the dish and encasement material were of the same texture and color as the fecal pellets everywhere present. Further, the color of the cases varied correspondingly to the two food substances available; those on straw were usually straw-colored while those on dark leaves had a black sheen appearance. Under the conditions of the experiment these eggs hatched very well, yet snails showed a marked preference for laying eggs below the surface of the soil. The egg was placed in a small excavation and sealed with a cap; under this condition they were most inconspicuous. When dug from the soil they still had a distinct encasement which may indicate that the snail prepared a lining for the excavation. Another possibility is that the albuminous covering of the egg may permeate and cement the surrounding mud. It was not determined whether the cap molded over the opening of the egg case was of feces, but its texture differed from that of the surrounding mud. Preference for laying in the soil was demonstrated by placing molds of mud (1" x 3/4" x $\frac{1}{2}$") in the petri dishes, in addition to the usual leaf and straw materials. They were left for ten days and removed. It has been assumed that an egg count would have to be determined on the basis of young snails hatched, but as the mud squares dried slightly the egg-cases became more conspicuous so that a fair count of eggs could be made. During the ten-day period, ten, one and twenty eggs were laid on filter paper, straw and leaves, respectively, while more than 300 were laid in the mud. It is likely then, that in nature eggs are laid chiefly in the soil, which probably affords a maximum protection, at least against dryness. Thus far, eggs have not been observed in water.

Sugiura (28) observed that incubation of O. nosophora required 11-13 days and Abbott (35) noted this period to be 15 days for O. quadrasi. The incubation period was followed for a series of 467 eggs. The findings, represented graphically (Figure 6), are quite different from those previously reported. Although a few eggs hatched as soon as 12-15 days the majority required 17-30 days. Curiously there were two peaks of hatching, the first being between 16-19 days and the second between 24-27 days. A very high mortality rate occurred among young snails hatched in the laboratory. They did not survive well in the petri dishes where they were hatched, and the few that persisted did not show normal growth. It is possible that some food element was lacking in the environment.

Summary - Eggs of O. nosophora were incidentally observed on filter paper in petri dishes. They were laid individually and encased with snail feces. Although egg cases were prepared on decomposing leaves, straw or filter paper, distinct preference was shown for soil in which shallow excavations were made to receive the eggs. Following deposition a dome-shaped cap was made to seal the egg chamber. Although hatching began after an incubation of two weeks it continued for a period of 32 days, with peaks o maximum hatching occurring at 16-19 and 24-27 days.
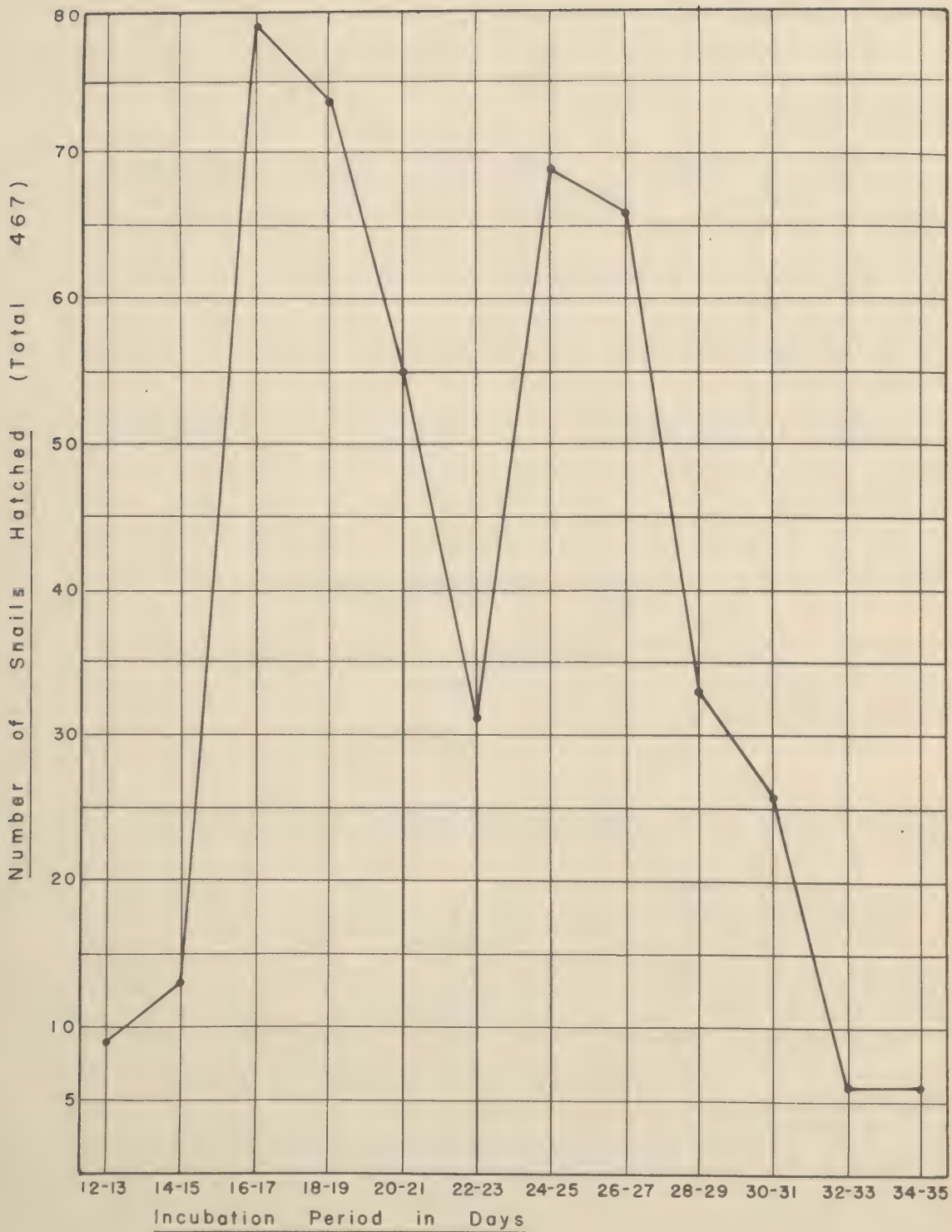
Figure 6. Incubation Period of O. nosophora Eggs

A COMPARISON OF THE SUSCEPTIBILITY OF O. NOSOPHORA AND O. FORMOSANA TO INFEC-
TIONS OF SCHISTOSOMA JAPONICUM FROM JAPAN: It has been shown that infections of
Schistosoma mansoni from distinct endemic centers of the world vary in their infec-
tivity for the several snail vectors involved. Conversely, susceptibility on the
part of a single snail species from diverse centers has been demonstrated. These
distinctions are commonly referred to as physiological, or strain differences.
Files and Cram (38) have presented clearly the inter-relationships of four strains
of S. mansoni and three species of vector from five distinct world areas. There
has been sufficient confirmation of their findings (39,40,41,42) to assume that this
phenomenon is real. A single report (43) has suggested that a similar situation
may exist for S. haematobium. The current investigation is an initial attempt to
determine if such differences occur in relation to S. japonicum and its several
vectors. O. nosophora and O. formosana collected in Japan and received from For-
mosa, respectively, were exposed to miracidia from infections occurring in Japan.

        Methods - Experimental snails were maintained on moist filter paper in
petri dishes, decomposed leaves being included as food. Those exposed to ten mir-
acidia were dissected before the resulting mother sporocysts lost their identity,
making it possible to determine the infectivity rate of the miracidia. On the
other hand, snails exposed to one miracidium were kept until the infections ap-
proached maturity.

        Results - Experiment 1: Two hundred specimens of each, O. formosana and
O. nosophora were exposed individually to one miracidium of S. japonicum (Table
XVI). Miracidia of human and dog origin were about equally used, and both watch-
glass and capillary pipette exposures were employed.

Table XVI. Susceptibility of O. nosophora and O. formosana
to S. japonicum of Japan.

EXPOSURES WITH A SINGLE MIRACIDIUM

No. Snails Exposed	No. Snails Surviving	Infection incidence (1) No.	%
		O. formosana	
200	194	0	0.0
		O. nosophora	
200	128	12	9.4

EXPOSURES WITH TEN MIRACIDIUM

Snails Exposed	Snails Surviving	Infection incidence (1) No.	%	Total Miracidia	Infectivity Rate (2) No.	%
		O. formosana				
135	126	1	0.8	1,260	1	0.08
		O. nosophora				
135	117	52	44.4	1,170	77	6.6

        (1) Determined by shedding 5 months after exposure;
             10% were crushed.

        (2) The infectivity rate is the ratio of mother sporocysts
             to total miracidia used.

Of 128 O. nosophora which survived the experiment 12, or 9.4%, were found to be infected. This was in contrast to the total absence of infections in 194 surviving O. formosana. The incidence for the former was determined by dissecting the snails, but to conserve specimens of O. formosana the occurrence of infection in this species was determined by placing specimens under conditions favorable for shedding; five months having elapsed following exposure. To check the negative results thus obtained about 10% of the snails were crushed; none were infected.

Experiment 2: One hundred thirty-five specimens of each snail host were exposed individually to ten miracidia hatched from dog or cow stools. Exposure was limited to the watch-glass method. Of 117 O. nosophora which survived, 52 (44.4%) were infected. This was quite in contrast to a single infection which occurred among 126 O. formosana. The occurrence of infection was determined, throughout the experiment, by dissection of snails. This was done approximately one month after exposure, allowing an enumeration of mother sporocysts, of which there were 77 in the 52 infected snails, an infectivity rate of 6.6% for the total number of miracidia used.

Discussion - The results of the above experiments show clearly that O. formosana and O. nosophora vary in their susceptibility to infection with S. japonicum as it occurs in Japan. In fact, O. formosana was almost completely refractory. The single infection which did occur in this species was very likely due to experimental exposure, as a preliminary check of the snails did not reveal infections and the age of the particular infection was the same as corresponding ones in O. nosophora. Nevertheless, the possibility of its being a natural infection cannot be ruled out. Further, there is the question as to whether it could have developed to maturity and produced cercariae. Under the conditions of this investigation the miracidia used were identical. The variation in their infectivity for the two snail hosts may well reflect specific differences of a kind which make O. formosana unsuitable for S. japonicum of Japan. Yet, since this snail is known to serve successfully as a host for the S. japonicum in Formosa there must also be a strain difference in the parasitic infection of the two countries. This parallels the reported diversity in S. mansoni and its snail hosts.

Summary - Oncomelania formosana and O. nosophora collected in Formosa and Japan, respectively, were experimentally exposed to miracidia from S. japonicum infections of Japan. A distinct difference in susceptibility was noted, O. formosana being almost completely refractory. Since the latter is an adequate host for the infection in Formosa it appears that S. japonicum, as it occurs in Formosa and Japan, entails physiological or strain differences.

DISTRIBUTION OF THE SNAIL INTERMEDIATE HOST OF SCHISTOSOMA JAPONICUM (ONCOMELANIA NOSOPHORA) ALONG THE TONE RIVER, HONSHU, JAPAN: This study was previously reported in considerable detail, including maps (9, 44). Only additional investigations made during the current year are reported here. As stated in 1950 the survey extended from Sakai to Sawara, a distance of at least 50 miles. In 1951 additional sectors of about 15 miles below Sawara and five miles above Sakai were surveyed; also sections of the Edo River were examined and Towa village in Saitama was searched for a third time.

Observations - Below Sawara, eleven carefully selected points on both sides of the river were examined, snails were found at eight of them. Over 100 specimens were collected at each of three of these places. Infections did not exist in any of the collections. These findings indicate that the section of the river bottom just below Sawara has one of the heaviest small populations of the entire Tone valley.

Downriver from Sakai for a distance of 15 miles, as previously reported, the sparseness of the vegetation on the river bottom and the sandy soil are unfavorable for O. nosophora; only a single collection of eight snails were found in this area. Similarly, the five mile sector just above Sakai was equally unfavorable, except at the level of Sakai on the south side. Here a limited area had a heavy growth of vegetation including numerous brush clumps, and a well established colony of snails was located about 400 yards above the water-gate of the auxiliary Edo River (which was originally the primary river below this point). This colony extends the known distribution of snails upriver about 12 miles. It was of additional interest since the origin of the colonies in

Saitama Prefecture may be geographically linked with it. If this inference is correct the origin of the Saitama colonies must have preceded dike construction along the Tone and/or Edo rivers; because the Naka River, where a focus of schistosomiasis is known to exist, has no direct connection with either of them, only draining lowlands outside their levees.

Whether snails exist farther up the Tone than Sakai is conjectural, but observations have been made at several points for a distance of 40-50 miles and in all cases the conditions of the river bed are unfavorable for O. nosophora.

At Towa village, Saitama, where the incidence of human schistosomiasis was shown to be 14% (24,45) intense searches for snails were made in 1949 and 1950 without success. In June 1951 the search was repeated and on this occasion snails were finally discovered inside the dike of the Naka River among dense reeds just above a swimming site. Three of 43 specimens were infected.

Thus far snails have not been located along the Edo River.

Summary - During 1951 information regarding the distribution of O. nosophora along the Tone River was significantly expanded. Numerous colonies were located over a distance of 15 miles below Sawara. Upriver at Sakai a well established colony was located immediately above the Edo River junction. The first snails to be found in Saitama Prefecture were discovered at Towa village.

IMMUNOLOGIC RESISTANCE AGAINST SCHISTOSOMA JAPONICUM RESULTING FROM INITIAL INFECTIONS: That immunologic responses to schistosome infections occur in the mammalian body is indicated by the positive reactions obtained with such diagnostic procedures as the complement fixation, precipitin and the skin tests. The possibility of immunologic resistance resulting from initial infections of Schistosoma japonicum is suggested by the fact that symptoms are more common among children, even when incidence and parasite density are relatively uniform for young and old. This is supported by limited experimental findings from dogs and monkeys. Currently, attempts have been made to demonstrate immunologic resistance to S. japonicum in hamsters and mice.

Methods - In the first experiment (9) eleven mice and five hamsters were exposed three times with intervening periods of three weeks; numbers of cercariae used consecutively for mice were 10, 20, and 40, while for hamsters they were 20, 100 and 200. The animals were autopsied and worms collected three to four weeks after the third exposure. If any immunologic resistance had been acquired from the first and/or second exposures, it might be expected that the worm burden of these animals would be less than the collective burdens of three controls receiving the equivalent exposures separately. In the second experiment, involving only hamsters, single exposures of 10 cercariae were initially made; then, after four months, definitive exposures (50 cercariae) of experimental and control animals were carried out to detect signs of resistance. Only 10 cercariae were used for the initial infections to assure animal survival; half the exposures involved cercariae from a single snail, while half were made with those of several snails. From the former, worms of a single sex might be expected, while mixed cercariae would account for both sexes and egg production. This differential was introduced for two reasons: (1) it was assumed that experimental animals would be more apt to survive infections in the absence of egg production, as they are the chief pathologic agent, and (2) it afforded the possibility of recognizing any additive effect of the egg as antigenic agent.

Results and Evaluation - A tabular presentation of data is given in Table XVII. In the first experiment 11 of 15 experimental mice survived, as did 42 of 45 controls. In the case of the hamsters the five experimental and 15 controls all survived. From worm counts at autopsy it was noted that only 31.3% of the total cercariae applied to experimental mice were recovered as adult worms, in contrast to 49.6% for the controls which had not previously been exposed. Considering the recovery for the control animals as 100%, the difference between them and the experimental

Table XVII. Infectivity of <u>Schistosoma japonicum</u> Cercariae
for Animals Previously Infected, as Compared
With Uninfected Controls

	Number of Animals Autopsied	Total Cercariae Used	Number Worms Recovered	Percent of Cercariae Recovered as Adults	Reduction in Worm Burden (Percent)
		Experiment No. 1			
Mice					
Experimental	11	770	241	31.3	36.9
Controls	42	930	461	49.6	
Hamsters					
Experimental	5	1600	410	25.6	27.5
Controls	15	1600	565	35.3	
		Experiment No. 2			
Hamsters					
Cercariae from 1 snail	10	600	247	41.2	32.0
Cercariae mixed	10	600	228	38.0	37.3
Controls	10	500	303	60.6	

Experiment 1 - Three exposures for experimental
animals.

Experiment 2 - Two exposures for experimental
animals.

animals is equal to 36.9%; this has been designated as reduction in worm burden, presumably due to immunologic resistance on the part of the host. For the hamsters the results were comparable but the corresponding worm burden reduction was only 27.5%.

In the second experiment, involving only hamsters, animal survival was good; only one experimental and one control were lost. The percentage of cercariae recovered as adults in the controls was 60.6. In contrast, the figure was 41.2% for hamsters which were exposed initially to cercariae of a single snail, and for those where mixed cercariae were used 38.0% were recovered as adults. These differences constitute a reduction of 32.0% and 37.3% for the two series, respectively.

Under the conditions of these experiments initial infections of S. japonicum in mice and hamsters seemed to afford some protection against subsequent infections. This assumption is strengthened by the fact that results were similar in the two experiments, the worm burdens for experimental animals in both instances being about one-third less than control animals. In this connection, Watts (46) noted a comparable reduction in incidence as well as worm burden in mice which were injected repeatedly with adult worm antigen of S. mansoni and subsequently exposed to this schistosome; furthermore, mortality rate was less in experimental animals as compared with controls.

The reduction of worm burdens was similar for hamsters infected with worms of a single sex (without egg production) and those infected with both males and females.

If our data reflects an immunologic response it would appear to be chiefly associated with the adult worm, rather than the presence of eggs in the tissues.

The repeated, heavier exposures, combined with a shorter immunizing period, and the lighter single exposures of the second experiment in combination with a longer immunizing period, were equally effective in reducing the worm burden of the subsequent exposures.

The experiment is being repeated a third time in order to accumulate data on sufficient animals to afford statistical significance. An attempt will also be made to show whether initial light infections can account for reduced mortality.

Summary - Partial immunologic resistance against S. japonicum appears to have been demonstrated in small mammals which had previously received light infections. Worm recovery was reduced about one-third in immunized animals as compared with controls without preceding exposures.

SEASONAL STUDIES OF SCHISTOSOMA JAPONICUM IN THE INTERMEDIATE SNAIL HOST (ONCO-MELANIA NOSOPHORA): Seasonal studies on propagation, growth and life span of O. nosophora, and the occurrence, maturation and duration of S. japonicum infections in the snail, have been previously reported (9, 24, 25). These studies have continued. Only one of three snail colonies studied in 1950 warranted further consideration (Mutsusawa). During the winter of 1950-51 a search was made for additional snail populations with a relatively high incidence of infection; furthermore, collecting points were sought which included all levels or conditions of infection maturation. The latter constituted an attempt to observe the entire span of the infection in a single season. Among seven to ten suitable collecting sites selected were several where only mature infections existed; in others both immature and mature infections occurred, while in one case only immature infections were present. Unfortunately, farmers applied a molluscacide to several of the more valuable colonies, but a good series was still available.

Collecting points were about equally divided between two types of snail habitats: (1) the terminal irrigation ditch which is the most common, and (2) the bases of hillside terraces which support rice paddies. In the latter cases snails move uninterruptedly into the paddies in late summer when the rice affords shade.

PROPAGATION, GROWTH AND LIFE SPAN OF SNAILS: In the summer of 1950, young snails appeared in abundance in mid-July collections at three colonies (Table XVIII). In one instance their presence was evident in June (Mutsusawa A), which was unusually early, while in two colonies (Mutsusawa B, C) reproduction was minimal throughout the summer. Observations in 1951 were distinctly different in that propagation was noticeably delayed. Whereas from 63% to 75% of the snail population at Shimosanjo, Aburakawa and Mutsusawa A in mid-July 1950 were young snails, only one of eleven collecting points (Shiozaki A) had such a percentage in mid-July of 1951. Propagation was not evident initially in several colonies until the mid-August collection, while for some this was not true until September. Even in October there seemed to be an increase of young snails in certain populations. These observations are consistent with those of McMullen (47). A possible explanation for this difference in the two seasons was the occurrence of rainfall during the month of May and possibly April. From early June until late September irrigation waters keep the snail environment continuously wet, so that rainfall, during that period, probably assumes a secondary role in the well-being of the snail; however, during April and May adequate moisture for snail activity is dependent on rainfall. During May of 1951 rainfall was limited to about two inches, half of which fell on the 8th of May, the remainder being so divided as to be of limited effect. Dryness was further increased by a lack of rainfall in the latter part of April. For preceding years the May rainfall was as follows: 1950 - 3 inches, 1949 - 3 inches, 1948 - 1 inch, and the years 1897-1926 - 4 inches average.

Table XVIII. The Occurrence of Young Snails in Monthly Collections*

	Mar.-Apr. %	May %	June %	July %	Aug. %	Sept. %	Oct. %
1950							
Shimosanjo		1	1	74	20	3	1
Aburakawa		1	0	75	24	7	
Mutsusawa A	29	20	51	63	22	16	23
Mutsusawa B	30	12	25	15	16	5	
Mutsusawa C	13	5	18	4	11	2	5
1951							
Fujimi A	1	3	5	1	14	17	15
Fujimi D	1		1	2	4	15	28
Fujimi P	2	1	0	23	40		
Sancho B	0		0	13	14	11	3
Ojimo C	16	10	9	3	24	20	28
Takizaka A,B,C	4		3	13	28	39	22
Yamanokami		5	3	6	9	29	18
Kyuji					4	14	22
Yamamoto E	8	2		1	1		
Yamamoto F			1	0	2	27	16
Shiozaki A				73	26		

* All snails measuring less than 6 mm. are designated "young".

For young snails with a size mode of 4 mm. to appear in mid-July collections, hatching would have to occur in early June (28), and egg-laying about three weeks earlier. Undue dryness in May, as was the case in 1951, would in general certainly delay laying.

Not only was propagation delayed in 1951 but it was also somewhat reduced, when compared with figures of 1950. Even in Sancho B, where young snails in small numbers were first noted in July, their occurrence was low throughout the summer. Only at Shiozaki A, where standing water existed even during May, did young appear early and in abundance. A low level of propagation may have been linked with the rainfall of 8 May 1951, which was referred to above. This is approximately the time when egg-laying would have to occur for young snails of 3-4 mm. to appear in July collections. Laying may have occurred at the time of the freshet of 8 May, only to have the eggs destroyed by ensuing dryness.

Observations on the life-span of O. nosophora are difficult because each successive generation can attain the size of its predecessors in the summer of its origin. Occasionally a colony fails for a season to produce offspring. In some cases the persistence of an existing population can be noted by their schistosome infections, even though diluted with the uninfected offspring. These conditions both existed in Mutsusawa B and C, propagation having been minimal for two summers. Young snails noted there in March-April 1950 were certainly hatched in late summer, 1949, while snails 6 mm. and over may have appeared earlier in the same summer, or in 1948, and even before. There was no explanation for the limited reproduction in 1950, but in May 1951, an application of a molluscacide greatly reduced the population, and eliminated a disproportionate number of the infected snails. In August of 1951, the remaining snails were at least two years of age and some may have been older. McMullen (47) also noted snails persisting two years, while Sugiura (28) recovered a few painted ones after five years.

Table XIX. Seasonal Studies: Infection of S. japonicum Identified with Snail Size

YAMANOKAMI

Snail Size (mm)	May '50 No. Snails	b-d	e-g	h	June No. Snails	b-d	e-g	h	July No. Snails	b-d	e-g	h	August No. Snails	b-d	e-g	h	September No. Snails	b-d	e-g	h	October No. Snails	b-d	e-g	h
8-9	78	2		15	114	1	7	36	64	8	2	6	102	1	6	46	63			39	37		2	18
7	147	6	1	35	110		5	18	111	16	4	24	102		8	45	70			41	118		3	39
6	63	1		16	15			2	35	8		2	28	1	5	12	52		3	11	88	1	1	31
5	12			2	5		1		13	1		2	12		5	6	39			5	33		1	7
2-4	2	1			3			1	1		1		10				35		1	2	20			
Total	302	| 78 - 25.8%			247	| 71 - 28.7%			224	| 73 - 32.6%			254	| 132 - 52.0%			259	| 102 - 29.4%			296	| 103 - 34.8%		

OJIMO C

Snail Size (mm)	Nov - Dec '50 No. Snails	b-d	e-g	h	Mar - Apr '51 No. Snails	b-d	e-g	h	May - June No. Snails	b-d	e-g	h	July - Aug No. Snails	b-d	e-g	h	Sept - Oct No. Snails	b-d	e-g	h
8-9	104	2	5	6	197		2	4	242		2	4	245			22	234			11
7	134	2	3	5	198		7	10	186	1	1	10	274	3		7	242	1		8
6	65	1	5	1	73	1	6	1	56		1	1	97			4	142			3
5	44	1	8		52		1	1	38			1	56				112			1
2-4	34	1			37				12				45				78			
Total	381	| 39 - 10.2%			557	| 34 - 6.1%			534	| 22 - 4.1%			717	| 36 - 5.0%			808	| 24 - 3.0%		

FUJIMI A

Snail Size (mm)	Mar - Apr '51 No. Snails	b-d	e-g	h	May - June No. Snails	b-d	e-g	h	July No. Snails	b-d	e-g	h	August No. Snails	b-d	e-g	h	Sept - Oct No. Snails	b-d	e-g	h
8-9	121		8	8	101		5	12	76				84		3	5	85		1	6
7	169	1	7	13	171		2	15	115				202	1	6	12	402	1	2	22
6	14	1	1	1	35			1	14				44		1	3	175			5
5	2				12				1				15				76			
2-4	2				2								37				50			
Total	308	| 39 - 12.7%			321	| 35 - 10.9%			206	| 10 - 4.9%			382	| 31 - 8.1%			788	| 37 - 4.7%		

SANCHO B

Snail Size (mm)	Nov – Dec '50 No. Snails	b-d	e-g	h	June '51 No. Snails	b-d	e-g	h	July – Aug No. Snails	b-d	e-g	h	Sept – Oct No. Snails	b-d	e-g	h
8-9	194	4	18	8	279		2	13	207	1	1	5	373	1		15
7	115	1			30				186	1		9	330		1	1
6	4								113		1		41		1	1
5									49				51			
2-4									30				13			
Total	313	31 – 9.9%			309	15 – 4.9%			585	18 – 3.1%			808	18 – 2.2%		

TAKIZAKA C

Snail Size (mm)	April '51 No. Snails	b-d	e-g	h	July No. Snails	b-d	e-g	h	August No. Snails	b-d	e-g	h	Sept – Oct No. Snails	b-d	e-g	h
8-9	38	2			60	1	1	3	15		3		55	1		10
7	70	3	4	3	134	1		9	31		5		106	3		18
6	49				48			3	23	1	3		87		3	
5	6				14				13	1			58		1	
2-4					23				31				39			
Total	163	12 – 7.4%			279	18 – 6.5%			113	13 – 11.5%			345	36 – 10.4%		

MUTSUSAWA

Snail Size (mm)	Mar – Apr '50 No. Snails	b-d	e-g	h	May – June No. Snails	b-d	e-g	h	July – Aug No. Snails	b-d	e-g	h
8-9	289	2	8	26	228	6	6	13	195		2	48
7	216	10	3	11	189	7	2	6	268		9	40
6	63	2	2	1	67	9	2	3	116		1	12
5	40	8	2		34	6	2	2	50	1		1
2-4	132	3	1		61	7	1		33		1	
Total	740	79 – 10.7%			579	70 – 12.1%			662	115 – 17.4%		

Snail Size (mm)	Sept – Dec No. Snails	b-d	e-g	h	March '51 No. Snails	b-d	e-g	h	May '51 No. Snails	b-d	e-g	h
8-9	229		4	47	11			2	85		1	8
7	291		1	41	65		1	7	91		1	2
6	92		1	8	42			3	23			
5	20				4				8			
2-4	15				2				8			
Total	647	102 – 15.8%			124	13 – 10.5%			215	12 – 5.6%		

* Stage a. Rarely seen
Stage d. Marks the appearance of germ balls in daughter sporocysts.
Stage g. Marks the appearance of the first few motile cercariae.
Stage h. Mature infections

a – 2 wks	e – 7 wks
b – 3 wks	f – 8 wks
c – 4 – 5 wks	g – 10 wks
d – 6 wks	h – 20 wks

Adapted from Faust and Meleney by McMullen (48)

FREQUENCY OF S. JAPONICUM INFECTIONS IN O. NOSOPHORA FOR THE KOFU VALLEY: For the Kofu Valley as a whole, the incidence of S. japonicum in the snail intermediate host is probably not much more than 2%. The higher infections of 5 to 50% shown in Table XIX are exceptional and as previously emphasized (9) are virtually "spots" in larger populations for which the frequency is 1 to 2%. Unless exposures are recurrent from season to season, a high incidence will be abruptly lowered by the appearance of a new uninfected generation and/or a high mortality in the infected population.

Regarding the recurrence of exposures from year to year at collecting points with a high incidence, it may be noted in Table XIX that new infections during 1951 were limited essentially to Yamanokami, with a few appearing in the Sancho B colony. This lack of recurrence, in conjunction with a spotty appearance of high rates of infection, suggests the possibility that exposures are the result of a combination of fortuitous circumstances, e.g., defecation by man or reservoir host in the vicinity of a snail colony where rainfall, should it occur before the stool dried, could wash the schistosome eggs into the environment of the snail. Application of night soil to field and gardens might fulfill one of these circumstances. Yet if this were the usual source, widely distributed as it is over the soil, snail infections would be less apt to be localized.

TIME OF OCCURRENCE, MATURATION AND DURATION OF SNAIL INFECTIONS: New infections in significant numbers were limited to Yamanokami (Table XIX). In June 1951, young infections in this snail population were of stages e, f, and g, which essentially cover that period of development from the germ ball stage of the daughter sporocysts to the time when the first few cercariae acquire motility. In July a considerable number of younger infections (c and d) sufficient in themselves to account for an incidence of 15%, were in evidence. Exposure accounting for these must have occurred about 15 June. Immature infections (e,f,g) were present again in August. These may have been the same infections noted in July as c and d, but it is possible that the latter had reached maturity by August and that the young infections of that month were of still another exposure, occurring as much as a week before the mid-July collection. The latter inference is supported by a marked increase in the overall incidence of infections, particularly mature stages, for the August collection and by observations on laboratory infections at summer temperatures. Furthermore, development of c and d stages found in mid-July to only e, f, and g stages by mid-August is untenable, as similar development occurred between May and June collections at Yamanokami under considerably lower temperatures. In September immature infections were almost entirely absent while a few e and f stages were present in October, presumably from another exposure. Thus it is possible that snails were exposed on as many as three different occasions during the summer at this colony. A cow, observed grazing in the vicinity, possibly explains the high incidence and repeated exposures. Relative to the re-occurrence of infections, it is interesting to note that four of the six colonies shown in Table XIX included numerous immature infections which had passed through the hibernation period of 1950-51. It can be safely assumed that they occurred either in late August or early September 1950. In contrast, very few immature infections were present in any of the colonies in the October 1951 collections; it is expected that this will be the status when collections are resumed in the spring of 1952.

Valuable observations were made with regard to the duration of infections in the current series of colonies. This was primarily possible because of the fact that reproduction was minimal and relatively late in the season. In addition, new infections were infrequent for all except one population. For the several colonies where collections were made in the fall of 1950 (Ojimo C, Fujimi A, Sancho B and Mutsusawa B, C) there was a marked drop in incidence of infections over the winter period. During the summer months the infection rate remained fairly constant without the addition of new infections, which is in contrast to the contention of McMullen (48) that infections present during late spring would be exhausted by the end of summer. The infections at Mutsusawa persisted from the fall of 1949, when about 50% were immature, until May 1951.

Some new infections during 1950 appeared to have occurred, but old ones obviously persisted throughout the summer and continued during the winter (Table XIX). This colony was spoiled in May 1951 by a molluscacide. The chemical was applied only a few days before the May collection and due to dryness had not yet killed many snails. In order to follow the infections longer positive snails of that collection were segregated by shedding and maintained under laboratory conditions until late August and early September 1951, at which time they were crushed. Observations were made on eight snails in which the sporocysts were thin. The infections appeared to be exhausted and about 50% of these infections could be traced to a time of origin in late August or September of 1949. How much older the others were could not be determined. On the basis of current observations it appears evident that infections can persist through two winters and probably a second summer.

Summary - Propagation of snails (Oncomelania nosophora) in the Kofu area was retarded and reduced in most of the populations studied during 1951 as compared with those of 1950. In this relation low rainfall in the latter part of April and May is believed to have suppressed egg-laying. Due to the absence of propagation in one snail colony it has been possible to recognize a life span of at least two years for O. nosophora. In one colony where the incidence of infections ranged from 25-50% the occurrence of immature infections indicated that as many as three separate exposures may have occurred during the summer; otherwise, exposures were minimal in the several populations sampled. For all collecting sites the data strongly suggest that schistosome infections in O. nosophora may persist for as long as two years.

DEPARTMENT OF PATHOLOGY

During 1951, the Pathology Department was faced with a variety of problems. In addition to continuing many of the studies of the previous year, the appearance at intervals of a group of infectious diseases in the Far East Command, particularly in Korea, required intensive study and aroused a great deal of interest. Various laboratories in the theater were visited, training and orientation of medical officers was continued, educational material in the form of CPC's were distributed, and close liaison was maintained with the various organizations in the theater. Much of the material reported upon was available because of the efforts and interest of medical officers elsewhere. In Japan during the first part of the year when medical facilities were taxed to the utmost and in Korea almost throughout the year, the collection of material and available information was an achievement requiring considerable sacrifice of time and effort during periods of stress. To these contributions must be added the work of many medical officers who during the year were on duty in the Pathology Department. This report is an acknowledgment of the work of all these medical officers.

ROUTINE SPECIMENS RECEIVED: During 1951 a total of 8,585 specimens were received in the Pathology Department as follows:

> Human Autopsies 807
> Surgical Specimens 7206
> Miscellaneous 571
>
> Total   8584

When compared with 1950, this represents an increase in total specimens of 86%, in autopsy specimens of 47%, in surgical specimens of 96%, and in miscellaneous specimens of 48%.

The following gives the work performed by the technical sections:

> Paraffin blocks prepared ... 25,912
> Routine H & E stains 53,224
> Special stains 7,370
>
> Total   86,506

During 1951, protocols, slides, paraffin blocks and wet tissues of 750 autopsies and 3,004 surgical specimens were sent to the Armed Forces Institute of Pathology.

Autopsies - As a rule autopsies were completed by the pathologists within 30 days but there was an additional 2 week delay for clerical work. However, by 31 January 1952 all but 15 autopsies received during 1951 had been typed or mimeographed and distributed.

Autopsies were received from Japan, Korea, the Philippine Islands and Okinawa. Members of the Pathology Department performed 28% of the autopsies. Approximately 49% of the deaths occurred in Japan and 45% in Korea. Table I lists the installations performing these autopsies.

The causes of death in these 807 autopsies are grouped in Table II and are given in more detail in Table III.

The apparent increase (20%) in non-battle injuries compared to 1950 was probably due to heightened activities and increased number of personnel in the theater. The increase in deaths due to disease (164%) was in part due to the inclusion of 162 autopsies on POW's in Korea and in part due to the outbreak of Epidemic Hemorrhagic Fever. Excluding the POW autopsies, there was a 55% increase in deaths due to disease in 1951.

Table I. Organizations Performing Autopsies in 1951

Japan Logistical Command 398
    406th Medical General Laboratory 226
    Osaka Army Hospital 47
    141st General Hospital 3
    279th General Hospital 3
    382nd General Hospital 5
    U.S. Army Hospital, 8162 A.U. 69
    U.S. Army Hospital, 8163 A.U. 10
    U.S. Army Hospital, 8164 A.U. 7
    U.S. Army Hospital, 8165 A.U. 7
    U.S. Army Hospital, 8166 A.U. 9
    U.S. Army Hospital, 8142 A.U. 3
    Johnson Air Force Base 2
    Nagoya Air Force Base 7
Korea 361
    1st Medical Field Laboratory 95
    2d Medical Battalion 1
    7th Infantry Division 1
    3d and 14th Field Hospital (POW) 12
    64th Field Hospital (POW) 36
    11th Evacuation Hospital 9
    21st Evacuation Hospital 48
    22d Evacuation Hospital 51
    25th Evacuation Hospital 1
    121st Evacuation Hospital 71
    171st Evacuation Hospital 1
    8055 MASH 7
    8063 MASH 7
    8076 MASH 11
    8209 MASH 2
    U.S. Navy Hospital Ship Consolation 8
Other FEC 48
    Rycom Army Hospital 29
    U.S.P.S.H. 5
    Clark Air Force Base 14

Table II. Summary of Causes of Death, 1950 and 1951

Cause of Death	1950	1951	1950	1951
Non-Battle Injuries			219	264
Accidental	196	200		
Suicidal	19	26		
Homicidal	4	17		
Circumstances Undetermined		21		
Battle Injuries			115*	90
Disease			148	391
Infants			51	39
Undetermined			14	23

   * Ten additional patients, listed under Disease also had Battle Injuries

    Among the autopsies on accidental deaths, compared to 1950, there was a decrease in 1951 in vehicular accidents but an increase in accidental poisonings. Of the latter there was an increase of 14 cases due to methyl alcohol alone or in combination with alkaloids (total 15), an increase of 9 due to barbiturates (total 11) and an increase of 7 due to alkaloids (total 13). As usual, toxicological examinations were frequently performed where the cause of death was not immediately apparent (see Chemistry report). Table III shows that there was a slight increase in suicides and a considerable increase in homicidal deaths.

Table III. Causes of Death in 807 Autopsies in 1951

```
Non-Battle Injuries .................................................. 264

    Accidental: .................................................. 200
            Trauma, type not stated ...........................    5
            Train .............................................    2
            Vehicular .........................................   32
            Fall ..............................................   11
            Airplane ..........................................   16
            Gunshot wound .....................................   22
            Poisoning .........................................   55
                Ethyl alcohol ...............................  8
                Methyl alcohol ..............................  13
                Methyl alcohol and alkaloids ................  2
                Alkaloids ...................................  13
                Carbon monoxide .............................  6
                Barbiturates ................................  11
                Barbiturates and alkaloids ..................  2
            Electrocution .....................................    3
            Anesthetic ........................................    5
            Crushing ..........................................    4
            Burns .............................................    7
            Asphyxiation ......................................    1
            Drowning ..........................................   29
            Shell fragments ...................................    2
            Lacerations .......................................    1
            Air embolism ......................................    2
            Postoperative hemorrhage ..........................    1
            Postoperative death, cause undetermined ...........    1
            Traumatic spinal tap ..............................    1

    Suicidal: ....................................................   26
            Gunshot wound* ....................................   21
            Fall ..............................................    2
            Poisoning .........................................    3
                Nicotine ....................................  1
                Carbon monoxide .............................  1
                Cyanide .....................................  1

    Homicidal: ...................................................   17
            Gunshot wound .....................................   10
            Stab wound ........................................    3
            Multiple contusions ...............................    4

    Undetermined circumstances: ..................................   21
            Gunshot wound .....................................    4
            Poisoning, probable ...............................   14
            Trauma ............................................    3

Battle Injuries ...................................................... 90

    Head ........................................................... 20
    Neck ...........................................................  2
    Thorax .........................................................  6
    Abdomen ........................................................ 10
    Thorax and Abdomen .............................................  8
    Extremities .................................................... 17
    Multiple ....................................................... 24
    Burns ..........................................................  3
```

Table III. Causes of Death in 807 Autopsies in 1951 Continued

Disease 391

    Infectious: 259
        Tuberculosis 40
        Pneumonia, lung abscess, etc. 15
        Salmonella infections 27
        **Bacillary dysentery 29
        Amebic dysentery 3
        Enterocolitis, etiology undetermined 3
        Amebiasis, other than colitis 7
        Diphtheria 1
        Tetanus 2
        Relapsing fever 5
        Meningitis, purulent 11
        Septicemia, probable 3
        Epidemic Hemorrhagic Fever 60
        Infectious hepatitis 18
        Poliomyelitis 14
        Japanese B Encephalitis 2
        Encephalitis, etiology undetermined 2
        Infectious polyneuritis (Guillain-Barre) 2
        Smallpox 10
        Typhus, probable 1
        Malaria, falciparum, acute 1
        Paragonimiasis, cerebral with brain abscess 1
        Fever of unknown origin 2

    Neoplasms and leukemia: 19
        Carcinoma, gastric 6
        Carcinoma, bronchogenic 1
        Carcinoma, hepatic 2
        Carcinoma, primary site undetermined 1
        Carcinoma, embryonal, testis 1
        Chondrosarcoma, femur 1
        Chondrosarcoma, skull 1
        Neuroblastoma 1
        Astrocytoma, frontal lobe 1
        Hemangioblastoma, cerebellum 1
        Leukemia, acute 3

    Cardiovascular and renal: 79
        Arteriosclerotic heart disease 36
        Congestive heart failure 1
        Rheumatic fever 1
        Rheumatic heart disease 1
        Rheumatic heart disease and bacterial endocarditis 2
        Myocarditis, interstitial 3
        Pericarditis, purulent 1
        Polyarteritis 1
        Malignant hypertension 1
        Aneurysm, hepatic artery 1
        Cerebral hemorrhage 20
        Glomerulonephritis 3
        Toxic nephrosis 2
        Lower nephron nephrosis, non-traumatic 3
        Pyelonephritis 1
        Pyelonephritis, renal rickets 1
        Polycystic kidney disease 1

Table III. Causes of Death in 807 Autopsies in 1951 Continued

Alimentary and biliary: 17
   ***Perforated viscus with peritonitis 2
     Peritonsillar abscess 1
     Esophagitis, ulcerative 1
     Volvulus, congenital anomaly of mesentery 1
     Cholangitis with liver abscesses 5
     Liver abscess, secondary to appendicitis 1
     Cirrhosis 4
     Fatty metamorphosis of liver 2

Miscellaneous: 17
   Diabetes 2
   Status asthmaticus 1
  ****Acute sickle cell crisis 3
   Pancytopenia 3
   Beriberi and other nutritional diseases 5

Infants 39
   Stillbirth 13
   Prematurity 7
   Congenital Anomaly 6
   Pneumonia, asphyxia neonatorum, atelectasis 5
   Hemorrhagic disease of newborn 2
   Erythroblastosis fetalis 1
   Shigellosis 1
   Septicemia 1
   Pyelonephritis 1
   Undetermined 2

         * One patient also had Epidemic Hemorrhagic Fever
        ** Includes 2 patients who also had Salmonella fever
       *** 11 deaths due to perforated typhoid-paratyphoid ulcers
            are included under Salmonella infection
      **** 2 of these patients also had Epidemic Hemorrhagic Fever

      Smallpox, epidemic hemorrhagic fever, infectious hepatitis, relapsing fever, and
the deaths in POW's will be discussed separately in this report. The case of aneurysm
of the hepatic artery with rupture into the hepatic duct was unusual. Survey of the
literature indicated that less than 100 such cases have been reported. As in other
years, malignancies were not frequently the cause of death because of the policy of ev-
acuating patients with chronic disease to the Z.I. Table IV compares the incidence of
the more frequent causes of death listed under disease for the years 1950 and 1951.

Table IV. Comparison of Incidence in 1950 and 1951
of Major Causes of Death by Disease

	1950	1951
Japanese "B" encephalitis	38	2
"Epidemic hemorrhagic fever"	0	60
Arteriosclerotic heart disease	37	36
Acute infectious diseases	12	115*
Cerebrovascular accidents	8	20
Infectious hepatitis	9	18
Poliomyelitis	10	14
Neoplasm	10	19
Smallpox	1	10

    * 83 cases were in POW's. There were also 40 cases of tuber-
      culosis and 10 miscellaneous infectious diseases in POW's
      not included in this figure.

Surgical Specimens - The majority of the surgical specimens consisted of the usual routine type of material. Table V gives the comparative frequency of the more common types of specimens submitted. In some cases the increase reflected only the increased personnel in the theater. However, the number of skin biopsies, gynecomastia and lymph node biopsies was greater than one would expect.

Table V.   Routine Surgical Specimens

	1950	1951
Appendices	935	1247
Endometrial biopsies	285	404
Cervical biopsies	243	358
Skin biopsies	401	1623
Nevi, benign	105	348
Breast, male	43	128
Breast, female	93	143
Pilonidal sinuses	80	61
Thyroid	34	76
Gallbladder	40	78
Lymph nodes	70	268

Among the 7,206 surgical specimens there were 229 malignant tumors, an increase of 64% over 1950. Some of this increase was only apparent since there was 96% increase in total surgicals and because of the considerable increase in personnel. The most notable increases were in the tumors of the skin, including melanomas, and of the genito-urinary tract. Table VI lists the malignant tumors received in 1950 and 1951.

Table VI.   Surgical Biopsies of Malignant Tumors, 1950 and 1951

Tumors	1950	1951
Skin and Mucocutaneous Junction:		
Basal cell carcinoma, skin	35	46
Precancerous (Bowens, Darier's)	-	3
Squamous cell carcinoma, skin, lip, etc.	20	34
Melanomas	2	10
Dermato fibrosarcoma protuberans	1	1
Hidradenocarcinoma	-	2
Breast:		
Adenocarcinoma	8	8
Adenocarcinoma, breast, metastatic to pleura, lymph nodes, etc.	-	2
Intraductal papillary carcinoma	2	-
Gastro-Intestinal System:		
Adenocarcinoma, stomach	1	5
Sarcoma, stomach	1	-
Adenocarcinoma, cecum	1	1
Adenocarcinoma, colon	2	2
Adenocarcinoma, rectum	3	-
Genito-Urinary System: Male		
Transitional cell carcinoma, bladder	3	1
Papillary carcinoma, bladder	1	3
Seminoma, testis	2	5
Seminoma, testis, metastatic	-	1
Embryonal carcinoma, testis	-	5
Teratocarcinoma, testis	-	5
Teratoma, adult type, testis	-	1

Table VI. Surgical Biopsies of Malignant Tumors, 1950 and 1951 Continued

Tumors	1950	1951
Genito-Urinary System: Female		
Carcinoma in situ, cervix	4	2
Carcinoma, infiltrating, cervix	2	7
Adenocarcinoma, uterus	3	2
Undifferentiated carcinoma, uterus	-	1
Leiomyosarcoma, uterus	-	1
Pseudomucinous cystadenocarcinoma, ovary	1	2
Papillary cystadenocarcinoma, ovary	2	2
Respiratory System:		
Transitional carcinoma, nasopharynx	1	-
Squamous cell carcinoma, epiglottis	-	1
Squamous cell carcinoma, larynx	2	1
Bronchogenic carcinoma	1	1
Bronchogenic carcinoma, metastatic, pleura lymph nodes	-	2
Neurogenous:		
Astrocytoma	-	2
Intracranial teratoma	1	-
Retinoblastoma	2	-
Retroperitoneal neuroblastoma	1	-
Hemangioblastoma	-	1
Lymphomas and Leukemias:		
Hodgkin's disease	8	7
Giant follicular lymphoblastoma	1	-
Lymphoblastic lymphosarcoma	1	2
Reticulum cell sarcoma	1	3
Lymphoblastic lymphosarcoma, with leukemia	1	-
Leukemia: Granulocytic	-	5
Lymphocytic	-	1
Unclassified	2	3
Lymphoma, unclassified	-	1
Mesodermal:		
Neurofibrosarcoma	8	4
Fibrosarcoma	1	2
Granular cell myoblastoma	3	-
Chondrosarcoma	1	2
Osteogenic sarcoma	2	1
Mixed sarcoma	-	1
Angiosarcoma	-	2
Undifferentiated sarcoma	-	4
Multiple myeloma	1	-
Miscellaneous Tumors:		
Mixed tumors, parotid	5	16
Papillary adenocarcinoma, thyroid	1	7
Hepatoma	-	1
Metastatic carcinoma, origin undetermined	2	7

Miscellaneous Specimens - The majority of these specimens were derived from animal sources and were submitted by other departments of this laboratory. Most of the specimens were either guinea pigs positive for tuberculosis or a variety of

animals used in attempted isolation of leptospirae. This latter group was studied during the early stage of the Epidemic Hemorrhagic Fever outbreak when it was suspected that these were cases of Weil's Disease. Field rodents and guinea pigs inoculated with human and animal tissues and excreta were examined. Leptospira were found in two naturally infected wild rodents. Tissues from 27 groups of animals including monkeys, rabbits, guinea pigs and mice inoculated with material suspected of containing the virus of Epidemic Hemorrhagic Fever were examined. No consistent reproducible lesions were recognized. Thirty-one animal specimens, including dog, cat, rabbit and mouse brains, were examined for rabies during 1951. Three specimens were positive for Negri bodies in contrast to 6 in 1950. One of the unusual miscellaneous specimens was a canine vaginal tumor which was transmissible and venereal. Table VII lists the types of miscellaneous material received.

Table VII. Miscellaneous Specimens, 1951

```
Animals for Tuberculosis: ........................   242
        Positive ...........................   241
        Negative ...........................     1

Animals for Rabies: ...........................    31
        Positive ...........................     3
        Negative ...........................    28

Animals for Smallpox: ...........................     5
        Positive ...........................     2
        Negative ...........................     3

Chorioallantoic Membrane for Smallpox: ...........     4
        Positive ...........................     3
        Negative ...........................     1

Animals for Schistosoma (Groups of animals) .....    12
Animals for Leptospira ........................   219
Animals for EHF (Groups of animals) .............    27
Miscellaneous ...........................    31
                                                    ——
                        Total              571
```

BATTLE INJURIES: Material obtained as a result of battle injuries fell into two large groups, surgical and autopsy. There was little duplication of patients in these two groups: few surgical specimens were submitted on patients who subsequently died.

Although there was 6 months more of combat in 1951 than in 1950 and more hospitals and personnel were involved, there was only a small increase in the number of surgical specimens resulting from battle trauma. An appreciable number of these resulted from frostbite which had occurred during the last two months of 1950 and the first two months of 1951. There was a decrease in the number of autopsies on patients suffering battle injuries. Obviously, only a small number of the total battle fatalities were autopsied. However, in both years almost all patients who suffered battle injuries and subsequently died in Japan were autopsied (97% in 1951). Available casualty figures bear out this finding that there was an actual decrease in the number of deaths of wounded patients who were returned to Japan. On the other hand, the autopsies from Korea represent only a sampling of the patients who died in hospitals there (3.2%). Table VIII lists the number of surgical and autopsy specimens showing trauma from battle injury in 1950 and in 1951.

Table VIII. Specimens Showing Trauma from Battle Injury

	1950	1951
Surgical specimens	208	249*
Autopsies	125	90**

    * Includes specimens from 9 POW's
    ** Includes 4 POW's who died of battle injuries

The surgical specimens show an increased number of amputations as a result of battle injury, but a decrease in most other types of specimens. This is compared in Table IX.

Table IX. Types of Surgical Specimens Resulting
from Battle Injury in 1950 and 1951

Site		1950		1951
Extremities		70		105
Battle injury	37		60	
Frost-bite	33		45	
Eyes		89		76
Central Nervous System		28		33
Lung and Pleura		-		12
Kidney		-		11
Spleen		9		7
Intestine		6		4
Arterio-venous Fistula		6		2

As in 1950, the central nervous system specimens were generally obtained during secondary debridement operations often to determine the presence of suppuration. One case was of particular interest. During operation a tissue nodule was found lying in the bullet tract. On section this proved to be a vascular meningioma which had been amputated by the missile.

The traumatized kidneys, lungs, pleura, spleens, intestines and arteriovenous fistulae showed nothing unusual. The eyes were not sectioned in this department but were submitted to the Armed Forces Institute of Pathology for study.

The following material had been accumulated on extremities amputated at the Tokyo and Osaka General Hospitals in 1950 and 1951: (a) aerobic and anaerobic cultures at various levels, (b) tissue content of antibiotics at the same levels, and (c) histologic sections from the same levels. Histories on these cases are incomplete and follow up is unavailable, except that apparently none of these patients died while in Japan. This material has not yet been correlated and studied. It will be the subject of a later report.

Frost Bite - During the winter 1950 - 1951, the Pathology Department received portions of extremities amputated because of frost bite with or without other injuries from 49 patients. There were several instances of multiple amputations including one quadruple amputation. These specimens consisted of 39 legs, the distal portion (tarso-metatarsal junction) of 17 feet, toes from 19 feet, one finger, and 2 hands making a total of 78 specimens. This report summarizes the clinical and pathological findings in cold injury and the limitations inherent in the study of material of this type.

Many of these patients received their injuries, both battle wounds and frostbite, during the retreat from the Yalu River and particularly from the Chosen Reservoir during a period of intense military activity. Clinical records were often incomplete and records of therapy were often lacking. Only those extremities amputated at the Cold Injury Center at Osaka Army Hospital and at Tokyo Army Hospital were submitted for study. Some patients had amputations of another limb performed in Korea. It was anticipated that other extremities would be amputated after evacuation to the Z.I. However, with few exceptions, attempts to follow these patients have been unsuccessful.

All patients were males. The available information concerning age and race are given in Tables X and XI but no conclusions can be drawn since exposure rates by age and race are unknown. Information is not available concerning past exposure to cold climates.

Table X. Age Distribution of Patients With Amputations For Cold Injury

Age	No. of Patients	Age	No. of Patients
17	3	25	1
18	7	26	4
19	6	27	4
20	5	28	2
21	3	33	1
22	2	35	2
23	1	36	1
		39	1

Table XI. Race of Patients With Amputations For Cold Injury

White 35
Negro 11
Oriental 2

Many of these men suffered severe hardship during the period of exposure. Often the exposure was prolonged and the patient could not tell when the cold injury had occurred during the period of exposure. Table XII therefore gives the estimated duration of the exposure resulting in cold injury in these cases. No correlation could be made between this duration of exposure and the degree of cold injury. Further, the types of exposure varied considerably. In some cases the men were pinned down in fox holes, others waded through streams. Some were captured and had their footgear stolen, and some had extremity wounds which were not treated for long periods of time. Some had to walk barefoot in the snow for days. A few had been unable to exercise their legs during a long truck ride. It is possible that some intentionally exposed themselves to cold injury to escape front line duty.

Table XII. Estimated Duration of Exposure

Time	No. of Cases
6 hours	2
8 hours	2
12 hours	4
1 day	14
2 days	5
3 days	3
5 days	1
8 days	1

There were 34 patients who suffered only cold injury and 15 who had, in addition, a battle wound of at least one extremity. The presence of a battle injury apparently determined to a considerable extent the type of surgical treatment, the time of amputation, and the extent of amputation. Since most of the foot amputations were performed through the distal third of the tibia, these are listed as leg amputations. Table XIII gives the number of patients with the various combinations for cold injury according to the presence or absence of battle injury and, for the group with battle injury, the number of amputated extremities which were the sites of such injury and the number of amputated extremities which had not received battle injuries.

Not only did the presence of battle injury lead to more radical amputation, but generally surgery was performed earlier. Of the 15 patients with battle injury, the

Table XIII. Amputations On Patients With Cold Injury With And
Without Battle Injury

Amputation	Total No. Of Patients	Cold Injury Only No. of Patients	Cold Injury and Battle Injury		
			No. of Patients	No. of Extremities with Battle Injury	No. of Extremities with Cold Injury Only
One leg	12	5	7	7	0
Two legs	12	6	6	7	5
Distal half, one foot	3	2	1	1	0
Distal half, two feet	6	6	0	0	0
Toes, one foot	6	6	0	0	0
Toes, two feet	6	6	0	0	0
One leg, distal half one foot	1	1	0	0	0
Distal half one foot, toes one foot	1	1	0	0	0
Finger	1	1	0	0	0
Two legs, two hands	1	0	1	1	3

estimated interval between the cold exposure and injury, and the amputation was avail-
able in 14 cases and was less than 3 weeks in 8. In patients without battle injury the
estimated interval was available in 26 and was less than 3 weeks for 9 patients. This
information is given in Table XIV.

Table XIV. Estimated Interval Between Exposure To Cold and Amputation

Interval in Days	No. of Patients	Patients Without Battle Injury	Patients With Battle Injury
2	1	0	1
13	1	0	1
14	2	2	0
15	2	1	1
16	4	2	2
17	4	2	2
18	1	0	1
19	2	2	0
21	1	1	0
24	1	0	1
25	2	1	1
26	1	1	0
28	1	0	1
29	1	1	0
30	1	0	1
31	1	1	0
32	1	1	0
33	2	1	1
35	1	1	0
36	1	1	0
37	1	1	0
41	1	1	0
42	1	1	0
44	1	1	0
45	1	1	0
46	1	1	0
60	1	1	0
64	1	0	1
87	1	1	0

Several factors were involved in explaining the differences between these two groups of patients. It is probable that patients with battle injuries of extremities exposed to cold were far more likely to develop severe cold injury. This, in part, may explain 8 bilateral amputations in which one of the extremities was not the site of battle injury (Table XIII). Further, some of these patients appeared much more toxic and clinically did not appear to be able, without danger, to wait out the period required for definite complete demarcation and mummification. Another factor, to be discussed in the separate report on battle injuries, was the presence of clinical findings suggestive of gas gangrene. In several of these patients crepitation was present and was the reason for early amputation. However, cultures from these extremities failed to reveal the presence of pathogenic clostridia.

The bacteriology of cold injury is deserving of special study. Only chance observations are available in this group. There were extremities showing advanced gangrene without clinical signs of clostridial infection from which clostridia including Cl. perfringens and Cl. tetani pathogenic for guinea pigs were recovered. Cultures were taken from 20 extremities and organisms were recovered from 18. Table XV lists the organisms recovered. Similar observations were made in extremities amputated for battle injury without frost-bite.

Table XV. Bacteria Recovered From 18 Extremities Amputated For Cold Injury

Organism	No. of Extremities With Positive Cultures
Hemolytic Staphylococcus	10
Non-hemolytic Staphylococcus	5
Alpha hemolytic Streptococcus	1
Beta hemolytic Streptococcus	6
Non-hemolytic Streptococcus	2
Proteus morgani	2
Paracolon species	2
Paracolobactrum aerogenoides	1
E. coli	2
Aerobacter aerogenes	1
B. subtilis, morphotype panis	1
Cl. sporogenes (non-pathogenic)	7
Cl. putrifactum (non-pathogenic)	2
Cl. perfringens (pathogenic)	2
Cl. tetani (pathogenic)	2

There was still another time factor involved in the amputations performed with short intervals from the time of exposure. Prior to 19 December 1950, there were 18 amputations for cold injury and battle injury. Of these 18 amputations, 17 were performed less than 3 weeks after exposure and one 21 days after exposure. Beginning 19 December 1950 there were 17 amputations for cold injury alone and 6 for cold injury and battle injury and of these, only one was performed less than 3 weeks after exposure to cold.

Except in the case of shortest duration (2 days) a line of demarcation was present in all specimens. In the amputations with shorter intervals the demarcation was broad and indistinct. As the interval increased, the demarcation between injured and uninjured tissue became abrupt and sharply defined with pink viable skin suddenly changing to mummified dead tissue or to an ulcerating foul liquifying band indicating the site of spontaneous amputation.

There is a suggestion in the histories that as time passed the final line of demarcation appeared to move distally so that there was some tissue recovery before the line demarcating viable from non-viable tissue became fixed.

* 10 for cold injury alone and 8 for cold injury and battle injury

Amputation was generally performed at the level of demarcation. This was true in all cases of amputation of toes. However, in the cases with battle injury and in some of the cases where an entire foot was amputated for cold injury alone, amputations were frequently made at a level above the ankle in order to assure a good stump for prosthesis. Of the cold injury alone, 25 had amputations at the level of demarcation and 8 had amputations above the line of demarcation. Of the cases with battle and cold injury, amputation was performed at the level of demarcation in one case and above the level of demarcation in 13 cases.

The microscopic changes were not different from those described by others. However, suppuration and inflammation were prominent and frequent. The reaction zone was broader than expected from the gross appearance and frequently extended distal to the cutaneous demarcation line. In this area vessels frequently showed considerable changes including endophlebitis, endoarteritis, thrombosis, and vasculitis. All types of inflammatory cells were present including eosinophiles. There was necrosis of muscle and fat. Sometimes the inflammation appeared to spare the corium. In the cases of shorter duration, the epidermis rapidly became thin, lost its rete pegs and resembled the margin of a chronic ulcer. In cases of longer duration such changes were much more abrupt.

In the mummified zone, inflammation was rarely seen. Medium and large vessels usually were widely dilated and engorged with blood. Some were not engorged and none showed evidence of true thrombosis even when a long time had elapsed between the exposure and amputation.

Sections of major vessels were taken at various levels beginning above the demarcation line and extending as far as the vessel could be traced. Above the demarcation line no changes could be detected but as this line was approached, intimal and sub-intimal proliferation appeared and the vessels appeared contracted with thick walls and plicated intima. The various vascular changes described above were seen in the reaction zone. Distal to the reaction zone, these vessels frequently appeared empty of blood, contained only the ghosts of nuclei and showed no evidence of any type of reaction.

No new evidence to support any of the various theories of the pathogenesis was developed in the study of these tissues. The impression was gained that "conglutination thrombi" when present reflected the tissue injury and were not the cause of such injury. The lack of any evidence of reaction in the mummified tissue, it was believed, indicated that injury due to cold had been progressive and occurred prior to the return of circulation.

Fatal Battle Wounds - The following two factors, types of missiles causing the wound and the interval between injury and death, were given in the 1950 report but when tabulated for this report were found to be not significant. Information was even less reliable in 1951 than in 1950 on the type of missiles involved in causing the battle injuries. Gunshot wound and shell fragment were common terms used for a variety of missiles. So many factors were involved in the interval between injury and death that no consistent pattern could be demonstrated. Variations up to 120 days were found.

Table XVI gives the anatomical areas involved in battle injuries and the number of cases for each area. Statistics are not available for the number of non-fatal wounds for each of these regions. However, it is known that extremity wounds were very frequent. In one mobile surgical hospital report, 37% of all battle casualties had extremity wounds only. Table XVI, it is believed, is misleading because a very small percentage of patients with extremity wounds died.

There frequently was injury to more than one anatomical area and it was not always evident which wound was the important factor in causing death. Table XVII gives

the immediate causes of death for the 215 cases. Some of the complicating causes such as Japanese "B" encephalitis were present only in 1950. Deaths due to secondary hemorrhage were found only in 1951. The 24 cases listed under miscellaneous causes of death were all single different causes of death.

Several of the immediate causes of death, such as brain abscess in head injuries and peritonitis in abdominal injuries, were directly related to the battle injury. Others were not so clearly related. Among these were lower nephron nephrosis and fat embolism which are discussed below.

Table XVI. Anatomical Regions Involved In Battle Injury

Region	1951	Total	% of total (215)
Head	20	56	26.0
Neck	2	2	0.9
Spine	-	8	3.7
Thorax	6	10	4.6
Abdomen	10	29	13.5
Thorax and Abdomen	8	20	9.3
Extremities	17	35	16.3
Abdomen and extremities	9	18	8.4
Abdomen and Spine	3	5	2.3
Miscellaneous (multiple)	15	32	14.9
Total	90	215	99.9

Table XVII. Immediate Cause of Death and Region Injured - 1950-1951

Cause of Death	Head	Neck	Spine	Thorax	Abdomen	Thorax and Abdomen	Extremities	Abdomen & Extremities	Abdomen & Spine	Miscellaneous	Total
Brain damage	20									2	22
Brain Abscess	12										12
Meningitis	8										8
Brain Hemorrhage				1			1			1	3
Subdural Hematoma	2										2
Japanese "B" Encephalitis	7						1				8
Pneumonia	1	4						1		3	9
Hemothorax				3		1					4
Pulmonary Embolism					2						2
Other Pulmonary	4		2	3						4	13
Secondary Hemorrhage		1		1	2	2		1			7
Traumatic Aneurysm		1					1				2
Peritonitis					9	6		4	2	4	25
Volvulus							2				2
Anesthetic							1	1			2
Lower Nephron Nephrosis	1				4	3	9	2	1	1	21
LNN and Pulmonary						1				1	2
LNN and Peritonitis					12	3		4	2	2	23
LNN and Gas Gangrene							2				2
LNN and Fat Embolism							4	1		2	7
LNN and Shock							2			1	3
Shock							3	1			4
Fat Embolism				1			6			1	8
Miscellaneous	1		2	1		4	3	3	10		24

Lower Nephron Nephrosis - Among the 125 autopsies of 1950 on patients who died of battle injuries there were 43 which showed evidence of lower nephron nephrosis (34.4%). Among the 90 similar autopsies of 1951 there were 35 which showed evidence of lower nephron nephrosis (38.9%). However, in many of these cases the lower nephron nephrosis was an incidental finding and not a direct cause of death. The lower nephron nephrosis was an important factor in causing death in only 21 of the 35 cases in 1951 (23.3% of 90), while in 1950 the lower nephrosis was probably important in 30 cases (24% of 125).

Interpretation of the rates given above must be made with caution. The incidence of lower nephron nephrosis in patients dying in Japan was accurate since autopsies were performed on almost all such patients. In Korea, autopsies were performed only on a small selected group of patients. The occurrence of lower nephron nephrosis in Korea where it was most likely to occur is therefore not known.

Even in the cases studied, clinical histories gave little information covering the period immediately after injury including information on the degree and duration of shock, the time elapsing between injury and treatment, and detailed data about blood transfusions.

The relation between lower nephron nephrosis and blood transfusion in patients suffering battle injuries was of course of paramount interest. Particularly important was the possibility of an increase in the incidence of lower nephron nephrosis because of the practice of using only group "O" blood for transfusion in Korea. The blood for transfusion was marked according to its Rh factor and as "high" or "low" titre. Patients in Korea with a blood group other than "O" received only "low titre", group "O" blood, frequently without cross matching. In Japan, patients were cross-matched and were given group and type specific blood.

Considering the many thousands of transfusions given in Korea, it was evident that serious transfusion reactions and associated lower nephron nephrosis must have been infrequent occurrences. In addition, factors such as deep and prolonged shock, extensive soft tissue trauma, generalized infections as peritonitis and localized gas gangrene and others, all of which are accepted as causes of lower nephron nephrosis, were present in many of these patients. They also increased the possibility of lower nephron nephrosis since they were the indications for multiple and repeated blood transfusions. The following discussion and tables gives the available information about these various factors.

There was a definite relation between the anatomical region injured and the occurrence of lower nephron nephrosis. Table XVIII shows that lower nephron nephrosis was much more frequently associated with wounds involving the abdomen and extremities than with other regions. This was even more apparent when tabulated by single regions, i.e. when more than one anatomical area was involved each was counted as a separate injury (Table XIX).

Twenty-seven of the 35 patients who showed evidence of lower nephron nephrosis in the 1951 series had an inflammatory or suppurative process at the time of autopsy. (Table XX). Some of these may have been related to the renal lesion although there was no reliable evidence of such a relationship in the histories available. The cellulitis infections in 1951 included 2 cases of clinical gas gangrene with recovery of pathogenic clostridia in both. In 1950 there were also two cases of gas gangrene. Cultures were performed in only one of these, resulting in demonstration of pathogenic clostridia.

Table XXI gives an estimate of the severity of the battle injury. Associated factors such as degree and duration of shock affect the estimation of severity of the injury. Severe shock was present in many of these patients. Obviously this was also the prime indication for blood transfusion. The two factors could not be separated as causes of lower nephron nephrosis on the basis of available information.

Table XVIII. Lower Nephron Nephrosis and Anatomical Regions Injured

| Region | 1951 | | Total 1950 - 1951 | | | |
	No. Of Cases	Cases with LNN	No. Of Cases	Cases With LNN	% of region with LNN	% of LNN
Head	20	2	56	3	5.3	3.8
Neck	2	0	2	0	0	0
Spine	0	0	8	1	1.25	1.3
Thorax	6	0	10	0	0	0
Abdomen	10	5	29	17	58.6	21.8
Thorax and Abdomen	8	2	20	9	45.0	11.5
Extremities	17	11	35	22	62.9	28.2
Abdomen and Extremities	9	5	18	9	50.0	11.5
Abdomen and Spine	3	2	5	3	60.0	3.8
Miscellaneous (multiple)	15	8	32	14	43.7	17.9
	90	35	215	78		

Table XIX. Incidence of Lower Nephron Nephrosis In Relation To Individual Areas Involved

| Region | 1951 | | Total 1950-1951 | | | |
	No. of Cases	Cases With LNN	No. of Cases	Cases With LNN	% of region with LNN	% of LNN
Head	24	2	64	5	7.8	4.4
Neck	6	2	6	2	33.3	1.8
Spine	5	3	25	8	32.0	7.0
Thorax	21	6	47	16	34.0	14.0
Abdomen	32	15	81	42	51.9	37.0
Extremities	35	21	72	41	56.9	36.0

Table XX. Inflammatory Processes Present at Autopsy - Cases With Lower Nephron Nephrosis Only

Inflammatory Process	1951	Total 1950-1951
Peritonitis	14	42
Pleuritis	2	13
Cellulitis	8	20
Pericarditis	0	5
Brain abscess	2	2
Meningitis	1	1

Table XXI. Severity of Battle Injury - Lower Nephron Nephrosis Cases Only

	1951	Total 1950-1951
Severe	26	58
Moderate	9	20

Table XXII. Degree of Shock in Patients With Battle In-juries - Lower Nephron Nephrosis Case Only

	1951	Total 1950-1951
Severe	18	36
Moderate	3	12
Not recorded	14	30

Twenty-three of the patients in 1951 and 64 patients in the entire group of 78 were known to have received blood transfusions, although in 7 cases the amount given was unknown. In 12 cases in 1951 (14 in the total group) there was no record of any blood transfusions. Table XXIII gives the available information concerning the amount of blood given. The average number of units of blood given in 1951 was 7.9 units (7.0 for total) with extremes of 2 and 28 (1 to 52 for total) and median of 6 (4 for total). In these cases, 57 patients were known to have been given 402 units of blood. However, not all of the blood was given in Korea. Information is not available concerning where these patients received their transfusions nor how much they received in Korea and how much in Japan.

Table XXIII. Transfusions Given To Battle Casualties - Lower
Nephron Nephrosis Cases Only

Units of Blood	1951	Total 1950-1951
1	0	7
2	3	8
3	2	10
4	3	7
5	2	4
6	1	2
7	3	4
8	1	3
9	1	2
10	1	1
11	0	1
13	0	1
14	1	1
17	1	1
23	0	1
24	1	1
27	1	1
28	1	1
52	0	1
Unknown	1	7
No record	12	14

In only one case in 1951 was a definite diagnosis of transfusion reaction recorded in the clinical record. This patient was transfused in Korea and was described as having had a severe transfusion reaction following debridement of severe extremity wounds. He remained in persistent "semi-shock" for the following 2 days. Five days later, 7 days after injury, he expired in a convulsive state. Autopsy study demonstrated lower nephron nephrosis and extensive fat embolism secondary to comminuted extremity fractures.

Although there were 35 cases with evidence of lower nephron nephrosis in 1951, only in 21 was the renal lesion an important factor as an immediate cause of death. In some cases a severe infection such as peritonitis or brain abscess caused death before the lower nephron nephrosis could produce marked azotemia. In other cases the lower nephron nephrosis was mild and may not have caused a fatal outcome. In 22 patients there was some evidence clinically of renal failure (Table XXIV.)

Each case was graded as mild, moderate or severe lower nephron nephrosis based on available clinical evidence and on the anatomical and microscopic evidence of renal damage. These were then compared with clinical diagnoses (Table XXV) as given in the histories. Most cases graded as severe were diagnosed clinically but, frequently, cases graded as moderate or mild were not diagnosed. This probably influenced treatment. An evaluation of therapeutic methods under field conditions, an important problem for clinicians, could not be attempted because the necessary information was not available.

Table XXIV. Clinical Evidence Of Nephrosis

	1951	Total 1950-1951
Azotemia	2	2
Oliguria	6	10
Oliguria and Azotemia	9	21
Oliguria and Hypertension	0	1
Uremia	3	15
Oliguria, Azotemia and Hypertension	2	3
Total	22	52

Table XXV. Comparison Of Estimated Severity Of Lower Nephron Nephrosis and Clinical Diagnosis

Clinical Diagnosis	Severe 1951	Severe Total	Moderate 1951	Moderate Total	Mild 1951	Mild Total
Lower Nephron Nephrosis	10	16	5	11	0	0
Possible L.N.N.	1	2	1	1	0	0
Uremia	3	5	0	0	0	1
Transfusion reaction	1	1	0	1	0	0
Not diagnosed	3	6	5	16	6	18

The interval between injury and death in the cases with lower nephron nephrosis contained many irrelevant factors. There were 6 who died more than 3 weeks after injury including one who died 100 days after injury. It was evident that in these 6, and some of the others, the lower nephron nephrosis did not originate at the time of injury. However, in the majority it was probable that the renal lesion occurred at the time of or soon after injury. Both in 1950 and in 1951 the median interval between injury and death was 8 days. In 1951, 71.4% and for the entire group 74.4% died 10 days or less after injury.

The pathology of lower nephron nephrosis has been described in detail and no new observations were made in these cases. The kidneys were almost always enlarged and above normal weight (Table XXVI). As in 1950, there was a patient in 1951 who had a unilateral nephrectomy and the remaining kidney weighed 225 grams. The average combined weight of the kidneys in the 1951 series was 451 grams (442 grams for the total) with extremes of 225 and 725 grams and a median of 450 grams for both 1950 and 1951.

Table XXVI. Weight Of Kidneys - Lower Nephron Nephrosis Cases Only

Combined Kidney Weight	1951	Total 1950 - 1951
200 - 300	3	6
301 - 400	6	16
401 - 500	11	27
501 - 600	6	18
601 - 700	1	1
725	1	1
Not recorded	7	9

The intensity of tubular epithelial necrosis was the most important single feature in estimating the severity of lower nephron nephrosis microscopically. Kidneys with prominent inflammatory reaction about necrotic tubules were also considered as severe cases. Although less attention was placed on pigmented casts, when these were numerous they affected the classification and tended to

place the case in a more severe grade. It was realized that lower nephron nephrosis is not a static lesion, but a process which undergoes progressive change through a state of damage to one of healing, that death can occur during any phase of this progression, and that an estimation of severity of involvement may be fallacious. However, the experience with this group of 78 cases suggests that it may be possible to establish criteria based on microscopic findings which will permit a rough estimation of severity of involvement. In practice, the estimations in these cases were made without reference to the history and then the correlations were made. With few exceptions the gradings based on microscopic findings were in agreement with clinical observations.

Fat Embolization - About the end of 1950 a study of fat embolization in deaths due to battle injuries was begun. A total of 76 cases have been studied to date. In all cases the lungs were studied for fat emboli. It was shown in 13 instances that the kidneys were negative when the lungs were negative. Thereafter kidney sections were not made if the lung sections did not show fat embolization. A total of 54 cases were examined for renal fat embolization.

It was hoped that information could be obtained on the following: (1) the incidence of fat embolization in patients dying from battle injuries regardless of the type of injury sustained; (2) the relation between fat embolization and the anatomical area injured; (3) the relation between fat embolization and the severity of injury; (4) the incidence of renal fat embolization; (5) fat embolization as a direct cause of death.

All tissues were fixed in formalin. Frozen sections of lung were cut at 25 micra and of kidney at 15 micra and were stained with Sudan IV. The severity of fat embolization was indicated by a one to four plus scale. One plus indicated that fat emboli were present in blood vessels but were scarce and difficult to find while four plus was used for severe fat embolization with emboli in almost every high power field.

Table XXVII gives the incidence and severity of fat embolization in the lungs of 76 soldiers who died of battle injuries and in the kidneys of 54 of these patients. Evidence of fat embolization was more likely to be found in the lungs than in the kidneys. In the entire group studied regardless of the type of injury, 32.9% had 2, 3 or 4 plus pulmonary embolization and 7.4% had 2 or 3 plus renal embolization.

Table XXVII. Incidence of Fat Embolization In Lung and Kidney

	No. of Cases	0	%	/	%	//	%	///	%	////	%
Lung	76	21	27.6	30	39.5	15	19.8	7	9.2	3	3.9
Kidney	54	31	57.4	19	35.2	3	5.6	1	1.8	0	0

The relation between fat embolization and the anatomical area injured is given in Table XXVIII. As would be expected, fatal extremity wounds were associated with a greater percentage of significant fat embolization (82.3%) than any other area. Thoracic, abdominal and thoraco-abdominal wounds showed significant pulmonary fat embolization in 4 of 20 cases (20%). There were no cases with significant pulmonary fat embolization among the 20 fatal cases of head and neck injury.

The severity of the injury showed in general little relation to the presence and severity of fat embolization. All of the head and neck wounds were severe yet none showed significant pulmonary fat embolization. However, an ambulatory patient who sustained a soft tissue wound of the right hand and fractures of the left carpal bones died under anaesthesia after manipulation of the fractures and repair of the lacerations. Sections showed 3 plus pulmonary fat embolization. Two patients without injury to bone also showed severe pulmonary fat embolization. One of these had severe injury of the intestine and peritoneal cavity and the other sustained burns of 90% of

Table XXVIII. Relation Between Severity Of Pulmonary Fat Embolization
And Anatomical Area Of Injury

Region.	No. of Cases	0	/	//	///	////
Head and Neck	20	6	14			
Extremities	17	3		9	3	2
Thorax	5		3	2		
Abdomen	8	4	2		2	
Thoraco-abdominal	7	7				
Multiple with bone injury	13			9	3	1
Pelvis	4	1	1	1	1	
Burns	2		1			1

his body surface as a result of a phosphorus grenade explosion. Table XXIX compares the severity of injury with the degree of pulmonary fat embolization.

Table XXIX. Comparison of Severity of Injury with Degree of Pulmonary
Fat Embolization

Degree of Injury	No. of Cases	0	/	//	///	////
Mild	4	1	2	0	1	0
Moderate	18	6	3	7	1	1
Severe	54	12	27	9	4	2

The incidence of renal fat embolization, as expected, was directly related to the severity of pulmonary fat embolization. When present it generally indicated severe fat embolization and the probability that fat embolization was an important factor in caus- ing the patient's death. However, the absence of significant renal fat embolization did not rule out fat embolization as a cause of death. Table XXVII gives the incidence and severity of renal fat embolization. Table XXX shows the relation between severity of pulmonary and renal fat embolization.

Table XXX. Relation of Pulmonary and Renal Fat Embol-
ization - 54 Cases

Pulmonary Embolization	Renal Embolization				
	0	/	//	///	////
0	13	0	0	0	0
/	21	2	0	0	0
//	5	3	1	0	0
///	2	2	2	0	0
////	0	0	1	2	0

Among this group of 76 autopsies it is believed that fat embolization was an im- portant factor in causing death or was the direct cause of death in 10 cases. Of these, 6 were associated with injury of bones, 2 with severe abdominal injuries, one with severe burns, and one with a wound of the thigh without bone injury but with severe burns. It is believed that injury of fat bearing tissues such as mesentery and subcu- taneous tissue can result in fat embolization although this is probably not frequently severe. In this group there were 30 patients (39.5%) who showed one plus pulmonary embolization. Neither clinically nor pathologically did this appear significant. It

did suggest that insignificant fat embolization may be of moderately frequent occurrence in patients dying of trauma. It has been shown by others that such slight degrees of fat embolization can occur even in deaths not associated with trauma. It is planned to continue this study. Further conclusions are not believed warranted at this time.

INFECTIOUS DISEASES: There were 259 deaths from infectious disease. Of these, 133 were in POW's. Although a high incidence of Japanese "B" Encephalitis was anticipated few cases were seen. On the other hand Epidemic Hemorrhagic Fever, Smallpox, and Infectious Hepatitis were encountered more frequently than expected.

Japanese "B" Encephalitis - Although JBE had been present in epidemic proportions during 1950, autopsies in only 2 fatal cases were received during 1951.

Smallpox - During January 1951, isolated cases of a fatal hemorrhagic and purpuric disease terminating in bullous exfoliation occurred in United Nations personnel recently returned from Korea. The possibility that these might be due to Epidemic Hemorrhagic Fever was considered and literature on this disease was reviewed. However, it was proven, by the virus department, that these were cases of Purpura variolosa. Subsequently other forms of smallpox appeared (see epidemiology section report). In all, the tissues from 10 autopsies were studied in this department. Deaths occurred during the period 10 January to 7 March. There were 5 Americans (3 military and 2 civilian), a British soldier, a New Zealander, a Turk, a Japanese and a Korean POW. Eight of the patients were exposed in Korea and two in Japan. Four of the cases were classified as Purpura variolosa, three as Variola hemorrhagica, and three as Variola simplex.

Certain anatomic features merited special note. The skin lesions determined the classification noted above. Those cases with characteristic umbilicated vesicles and pustules were called typical smallpox. Where there was hemorrhage into the vesicles, the diagnosis was hemorrhagic smallpox. Purpura variolosa was reserved for those fulminating cases which developed purpura without or prior to vesicle formation, the latter occurring terminally and consisting occasionally of large bullae. In these cases the characteristic smallpox eruption was lacking. Guarnieri bodies were demonstrated in all except one case. No skin was submitted for histologic study in this case but the diagnosis was confirmed by virus isolation. Besides the skin changes, characteristic lesions were found in the viscera. In all 7 cases of Purpura variolosa and Variola hemorrhagica, interstitial nephritis was observed. This consisted of a dense infiltration of mononuclear cells in the interstitial tissue about the cortico-medullary junction, streaming out to a lesser extent through the medulla. In some cases, this infiltrate was so prominent that it could be recognized by looking at the slide with the unaided eye. Accompanying this inflammatory change in 5 of the cases there was a degenerative change in the tubular epithelium characterized by granularity and swelling of cytoplasm, occasional desquamation of epithelium, and casts. A constant concomitant of the nephritis was hemorrhage about the renal pelves and calyces. One of these cases had squamous metaplasia of the renal pelves and urinary bladder. Squamous metaplasia of other epithelial tissues was somewhat more frequent. It occurred in the pancreatic ducts (3 cases), the Pituitary gland (2 cases) and in the trachea (1 case). Neither squamous metaplasia nor interstitial nephritis was observed in any of the non-hemorrhagic cases.

An attempt to correlate the kidney lesions with clinical signs of renal failure was unsuccessful. These lesions occurred only in the hemorrhagic and Purpura variolosa cases. In These cases the average length of illness from first symptom to death was less than 8 days, and in no instance exceeded 11 days. The general toxemia overshadowed whatever effects the renal impairment may have had. In three cases the urine was grossly bloody, and one patient became anuric a few days before death. It is likely that nephrosis was a contributory influence in all of these severe cases.

Epidemic Hemorrhagic Fever - During 1951 there was an epidemic of EHF. This section of the report deals only with the pathology and suspected pathogenesis of this disease - other sections (Bacteriology, Virology, and Epidemiology) should be consulted for additional information.

As was mentioned under the section on smallpox, the possibility of an outbreak of this disease was considered in January and February 1951 until the diagnosis of smallpox was established. In May a single case was studied and was incorrectly considered to be a case of purpuric and leukemoid reaction due to an unknown poison. Not until the end of June and the beginning of July were additional cases received and the correct diagnosis suspected. Ultimately, 60 autopsies showing the changes accepted as those of EHF were examined. Eight additional autopsies on patients who died in December were received during the first weeks of January, 1952. These last cases are included in Figure 1 but are not included in the discussion.

Because EHF represented a new disease in American medical experience and because there were no reports of the pathology in western medical literature, a resume of the findings was submitted for publication in the 1951 Yearbook of Pathology and Clinical Pathology at the invitation of General Elbert De Coursey and Dr. Howard Karsner. A more detailed description was prepared for inclusion with other papers in a symposium on EHF which is to be published in a journal not yet named.

The mortality rate in this epidemic was about 8%. In fatal cases, the course was generally short. The median duration of disease was 8 days with extremes of 2 and 29 days. In approximately 88% death occurred within 2 weeks of onset of disease. Shock and uremia were the most frequent causes of death with shock being most important during the first week of disease.

Clinically, the disease followed a characteristic pattern which could be confused with blood dyscrasias, relapsing fever, Weil's disease, acute glomerulonephritis, and malaria. Of these, Leptospirosis caused the greatest confusion. It was finally excluded when intensive efforts failed to reveal organisms in tissues, on culture or by animal inoculation, and when acute and convalescent sera uniformly failed to cause agglutination of a series of strains of leptospirae or to give a positive complement fixation test.

Grossly and microscopically, the pathologic changes were unusual and characteristic. Table XXXI gives the available weights of the important organs. The cortex of the kidneys was sharply demarcated from the congested and hemorrhagic medulla. This hemorrhage was most intense in the subcortical area and extended toward the papillae. In some cases there were areas of necrosis in the pyramids. Submucosal hemorrhage of the renal pelvis was seen frequently. Microscopically all fatal cases showed medullary hemorrhage and 67% showed small or large peculiar foci of necrosis in the pyramids. These resembled focal areas of aseptic infarction or bland necrosis but rarely showed any cellular reaction despite the fact that at least in some cases it was evident that these lesions were of considerable duration. Further, no evidence of vascular occlusion or obstruction was ever demonstrated. Many kidneys contained pigment casts in addition to the many other types of casts present, but here too, foci of inflammatory reaction were usually lacking. On the other hand, the frequent peripelvic and submucosal pelvic hemorrhages usually were associated with a non-specific inflammatory reaction.

Table XXXI. Weights Of Organs In Fatal Cases Of Epidemic Hemorrhagic Fever

Organs	No. of Cases	Weights of Organs in Grams	
		Average	Extremes
Kidneys*	46	521	300 - 900
Heart	46	357	200 - 500
Spleen	46	243	120 - 600
Liver	45	1868	1200 - 2650
Lungs*	41	1220	225 - 2340
Brain	39	1418	1250 - 1700

* Paired organs are recorded as total combined weight

Figure 1. Duration of Disease in 65 Fatal Cases of EHF

Necrosis, similar to that seen in the kidney medulla, was also found in 75% of the anterior lobes of the pituitary and 19% of the adrenals. In two cases the pancreas contained focal necrosis which appeared limited to the islands of Langerhans with some spilling over to involve adjacent acini. In two other cases the liver showed mild mid-zonal necrosis. In none of these sites was there evidence of vascular engorgement and hemorrhage in the vicinity of the necrosis.

Although the heart was enlarged in some cases, the most prominent finding was diffuse hemorrhage limited to the right atrium. Microscopically, this often showed no associated inflammatory infiltrate. Similar severe intense hemorrhages without cellular reaction were found in the submucosa of the gastro-intestinal tract, particularly the stomach. Submucosal hemorrhage has been described above in the renal pelvis, but there it was associated with an inflammatory reaction. Focal and petechial hemorrhages of larger or smaller size were frequently found beneath the mucosa of the urinary bladder, in subcutaneous tissue, in the posterior lobe of the pituitary, in the adrenals, in the pancreas, in the epicardium, and in the brain particularly in the hypothalamic area. Two patients had serious cerebral hemorrhages, one had a subdural hemorrhage and one patient died after spontaneous rupture of the spleen.

Microscopically, a peculiar mononuclear cell infiltrate in certain tissues was another characteristic of this disease. The infiltrate consisted of phagocytes, plasma cells, and large cells resembling immature granulocytes which could also be seen in vessels of many organs and in the sinusoids of the liver and spleen. In some instances, eosinophiles, polymorphonuclear leukocytes and mast cells could also be identified. Such infiltrates were frequently found in the left atrium, sometimes the right atrium and adjacent portions of the ventricles. They were found in the interstitial tissue of the pancreas and as a mild perivascular infiltrate in the superficial portion of the corium. They were frequently seen beneath the mucosa of the esophagus and when the latter was ulcerated were present deep in the tissues.

The spleen and lymph nodes consistently showed no germinal center activity but contained a variety of mononuclear cells. In a few cases there was definite evidence of extramedullary myelopoiesis in the spleen and in the liver. Bone marrow usually was hyperplastic with many megakaryocytes and some plasma cells.

Considerable discussion was held concerning the pathogenesis of these lesions. The possibility of a Truetta mechanism being involved in the renal lesion was discarded when it was noted that although only the juxtamedullary glomeruli contained blood in some cases, in an equal number of other cases all glomeruli were engorged. The shock-like state which occurred so frequently in patients who died of the disease could not be related to any consistent anatomical change. However, it was suspected that the pigmented cases and clinical evidence of lower nephron nephrosis was related to these episodes. Severe protracted vomiting which was almost always present was believed to be the cause of the esophagitis and certainly had an important effect in embarrassing acid-base and fluid balance controls. A search for inclusion bodies, rickettsia, leptospirae, bacteria and fungi was unsuccessful. The site of entrance of the infectious agent and the site of propagation of the agent was unrecognized.

Several cases had unusual features. Two patients who died while enroute to the hospital apparently complaining of only mild symptoms were found to have all vessels packed with sickle cells. It was suspected that these patients died in sickle cell crisis during the early stage of an EHF infection although it was also recognized that these could be instances of sickle cell trait made unusually prominent by terminal and postmortem anoxia and formalin fixation. The possibility that a circulating toxin present in EHF might cause precipitate sickle cell change was explored with negative results. Separate portions of red blood cells from a patient known to have sickle cell train were incubated with 5 acute and 5 convalescent sera collected from 10 patients who had EHF plus suitable controls. No differences were noted in reaction of the cells to the sera or the controls.

Although there was no evidence of primary or important liver involvement, it was noted that there were 3 fatal cases with chronic liver disease antedating the EHF. One patient who committed suicide showed the usual pathologic changes of EHF, although he apparently had very few symptoms.

It appeared probable that the disease in patients without hemorrhagic features would be recognized if a satisfactory diagnostic test were available. For this reason it was suspected that the name was a misnomer. The disease therefore has been coded in the Pathology Department as, "Infectious disease, etiology undetermined (Epidemic Hemorrhagic Fever)."

Finally, in an attempt to explain the varied clinical and pathologic observations a theory was suggested which so far has not been confirmed by laboratory or clinical methods. It was suggested that a living agent, virus or rickettsial, caused the disease, that either the agent or a toxin entered the circulation and produced a widespread effect with particular effect on capillaries and on hematopoietic tissues. It was further suggested that the toxic substance was concentrated in the renal tubules and thus produced a localized effect in the kidney. At the same time, in those patients with severe infections, there was sufficient toxic material to cause necrosis in particularly susceptible tissue such as the pituitary and adrenal. It was thought the hemorrhages in the right atrium and gastro-intestinal tract were related to strains and stresses during periods of vomiting just as subconjunctival hemorrhage had been observed to occur during episodes of vomiting. Obviously, considerably more work must be done before these problems will be solved.

Leptospirosis in Guinea Pigs and Hamsters - The Bacteriology Department conducted a search for leptospira in wild rodents trapped in Korea in the area where Epidemic Hemorrhagic Fever was prevalent during a period when that disease was suspected to be Leptospirosis. Approximately 100 animals were examined and their tissues were cultured for leptospira. Leptospira were isolated in two instances. A medical officer assigned to the Bacteriology Department conducted the following study of the pathology of Leptospirosis in guinea pigs and hamsters infected with subcultures from these two wild Korean rodents.

The pathological lesions of Leptospirosis in guinea pigs varied somewhat but, in general, characteristic lesions were found in all the animals. At autopsy the most constant findings consisted of pulmonary petechial hemorrhages and ecchymoses visible grossly through the pleura, and retroperitoneal hemorrhages along the course of the psoas muscles. Other gross findings were inconstant. The liver and kidneys were usually enlarged. Some were congested and dark while others were pale. Occasionally, the surface of the kidney was slightly granular and contained tiny, pin-point hemorrhages. The spleen showed no changes grossly. Except for one case with grossly bloody intestinal contents, the intestines were normal. Lymph nodes were not enlarged. There were hemorrhages in the connective tissues around the testis and epididymis but not in the parenchyma. Rarely, there was an excess of peritoneal serous fluid with a slightly sanguinous tinge. The adrenals were congested in some cases, but rarely contained any hemorrhages.

Microscopically, the lungs showed diffusely scattered areas of focal alveolar hemorrhage. Occasionally, this was confluent and extended through the section. Some of the animals showed slight thickening of the alveolar walls, some emphysema, but most showed areas of focal atelectasis. In no instance was there evidence of a pulmonary inflammatory process. Usually, but not always, leptospirae could be found in the lungs in the heavily infected cases more easily than in the lightly infected cases. They were most readily found in the pulmonary tissue surrounding areas of alveolar hemorrhage and not within the hemorrhagic areas. This may be due to the heavily stained and packed erythrocytes in the hemorrhagic areas which made recognition of leptospirae difficult. The organisms were never very numerous in the lungs. Usually a prolonged search was required to find them although this varied with experience in recognizing them.

The liver usually showed focal areas of hepatitis. The portal areas were infiltrated with mononuclear cells, a not unusual finding in normal guinea pigs, but the mononuclear cells streamed out along the interlobular septa in abnormal numbers. The areas of focal hepatitis were usually to be found around, or adjacent to, the central veins where there were some extra-sinusoidal mononuclear phagocytes and a few degenerating or necrotic hepatic cells. In some cases, the lobular pattern was lost and the areas of hepatitis followed no specific pattern. Under low power, the liver appeared quite cellular because of the increase in the number of white cells in the sinusoids. In three cases, small areas of anemic infarction in the subcapsular region were observed. Varying degrees of fatty degeneration were seen but this was never severe and was not correlated with the severity of the infection. Leptospirae were usually extremely abundant in the liver. Often the liver was so heavily infected that the organisms appeared to form a continuous, reticulum-like network lining the sinusoids throughout the section. Leptospirae, if present, were most likely to be found in and recovered from the liver.

The kidney lesions varied greatly in severity but were basically similar. Hemorrhage into Bowman's space was often seen but the degree varied greatly, not only from case to case but in the same section. The glomerular tufts often appeared abnormally cellular due to an excess of white cells in the glomerular capillaries. Some glomerular tufts were congested and swollen, others were contracted. Red cell casts and amorphous eosinophilic casts were numerous in the tubules of the cortex. Cloudy swelling almost invariably was present. It was most marked in the convoluted tubules where the swelling often occluded the lumina. Some of the convoluted tubules were dilated suggesting obstruction at a distal point. Only occasionally were small hemorrhages found in the parenchyma although hemorrhages, and occasionally inflammation, were quite frequent in the peripelvic connective tissues. Some of the kidneys contained small nonconspicuous areas of interstitial nephritis. Leptospirae could usually be found in the kidneys and sometimes were quite numerous. They were more numerous in the tubular casts than in the parenchyma but there was no predilection for any specific area.

Other viscera did not show constant lesions. The Malpighian bodies of the spleen were not distinct and both the white and red pulp were less cellular than normal. The sinusoids were relatively devoid of blood but not collapsed. Aggregations of red cells and clumps of amorphous eosinophilic material were seen in the sinusoids. The eosinophilic material resembled masses of phagocytized erythrocytes. The adrenals were congested but only rarely contained petechial hemorrhages. Leptospirae could be seen in some cases. Hemorrhages were occasionally seen in the connective tissue about the testes and epididymis but not in the parenchyma. The ovaries showed no changes. A hemorrhagic cystitis was noted in one case. Microscopically, hemorrhages were widespread in the submucosa and muscle wall but there was no evidence of inflammation. In the bladder leptospirae were seen only in the epithelial layer.

The extensor muscles of the thigh, the paravertebral muscles and the striated muscles underlying the retroperitoneal hemorrhages were not unusual. Rarely, there was a slight focal mononuclear cell infiltration in the loose connective tissue. Leptospirae were not seen. Vertebral marrow, vertebrae and the spinal cord were examined in two cases. Nothing remarkable was noted.

Hamsters presented almost the same lesions as guinea pigs. However, the kidneys of the hamsters showed more prominent changes which were quite characteristic. There was severe hemorrhage and considerable amorphous eosinophilic material in Bowman's spaces. The eosinophilic material appeared to be material of high protein content, including products of red cell lysis. Erythrocytes and the amorphous material formed numerous tubular casts. There was extensive degeneration of tubular epithelium. Numerous leptospirae were usually present.

In summary, the principal pathological lesions of Leptospirosis in guinea pigs and hamsters were found in the lungs, liver and kidneys. Alveolar hemorrhage was

constantly present. Focal hepatitis was usually present and leptospirae were most abundant in the liver. The kidneys showed hemorrhage into Bowman's space, red cell and hyaline casts, cloudy swelling of the tubular epithelium, tubular atrophy, and peripelvic hemorrhages. A peculiar feature was the marked change found in some organs, notably the lungs, with few leptospirae present. On the other hand, the liver which contained enormous numbers of leptospirae showed only minimal change.

Infectious Hepatitis - During 1951 a total of 273 liver biopsies were examined, the majority of which were submitted by the hepatitis center at the 8164th Army Hospital in Kyoto. Most of these biopsies were performed according to a preconceived plan and fell into two large groups. The first group of 84 were collected during the period 1 September 1950 to 1 June 1951 and the second group of 76 were submitted during the summer of 1951. During the first investigation, a total of 2,351 patients had been hospitalized at the hospital center for suspected infectious hepatitis (Table XXXII).

Table XXXII. Admissions To The Hepatitis Center 1 September 1950 - 1 June 1951

Infectious hepatitis	2,011
Serum hepatitis	19
Cirrhosis	4
Hemolytic Jaundice	7
No liver disease	301
Total	2,351

Among this large group of patients there were 73 who were considered to be unusual and for whom liver biopsy was believed indicated. Eighty-four biopsies were performed on these 73 patients for the reasons given in Table XXXIII.

Table XXXIII. Indications For Liver Biopsy

	Number of Patients	No. with Repeat Biopsies
Diagnosis	35	3
Prolonged disease	27	4
Severe disease	8	2
To assess therapy	3	2
Total	73	11

As a result of biopsy for diagnosis, the clinical opinion was confirmed in 13 (37%), a precise diagnosis not established by clinical methods was made in 12 (34%), and liver disease was excluded in 10 (29%).

An important indication for biopsy was to determine the future status of the patient and to decide whether he should be returned to duty, given additional convalescent care or returned to the Z.I. As a result of liver biopsies, disposition of patients whose clinical course and laboratory findings had been unusual, could be made with greater confidence. Table XXXIV. gives the disposition of these patients.

Table XXXIV. Disposition of 73 Patients Following Liver Biopsy

Evacuated to United States	14
Limited duty (to be re-evaluated)	18
From limited duty to full duty	3
To full duty (chronic disease excluded)	34
Still in hospital	4
Total	73

It was concluded that with proper evaluation, liver biopsy was a valuable diagnostic procedure, that its risks were not to be minimized, but that with proper care and precautions no injury was to be anticipated.

Pathologically a variety of lesions were encountered. Since these were all selected cases, generally with symptoms for a considerable period, no early lesions of hepatitis were seen. However, several specimens showed changes suggestive of residual scarring following an attack of hepatitis. Generally this was slight, and it would appear, could heal with little or no residual effect on liver function. Another feature of interest was the appearance of moderate fatty metamorphosis in 6 (17%) of the cases biopsied because of prolonged and/or severe disease.

Beginning in July 1951 the second investigation was started which was intended to check the histological changes associated with vitamin B_{12} therapy. A series of 80 patients apparently had had a shorter than expected clinical course when B_{12} supplement was added to the diet. It was decided to perform biopsies on a group of treated patients and a group of controls. The biopsies were to be performed as early in the disease as possible before treatment and again 28 days after the first biopsy. The clinical results will be reported by the hepatitis research team. Although the series of liver biopsies was not of value in the solution of the original problem other observations were made which were of considerable interest.

In order to avoid prejudice and the effects of chance only half the patients biopsied received B_{12}, otherwise all received the same treatment and diet. Similar duration of disease and severity of disease as determined by total serum bilirubin were the criteria used to place patients in the treatment and control groups. The pathologist did not know in which group the patient was until the investigation was completed. A total of 38 patients had liver biopsies during the investigation.

This study made available a group of biopsies early in the course of infectious hepatitis with a repeat biopsy 28 days later. The average duration of disease from the onset of first symptoms was 14 days, the median duration was 14.5 days, and the extremes were 8 and 24 days. Figure 2 illustrates the duration of disease in this group. Eight patients had a TSB (total serum bilirubin) range of 1 - 5 mgm%, 14 had TSB's of 5 - 10 mgm%, 9 had TSB's of 10 - 15 mgm% and 7 had TSB's of 15 - 20 mgm%.

There are few reports in the literature of liver biopsies taken during the early stages of infectious hepatitis. This group presented changes which had not been appreciated previously by members of the Pathology Department. The changes during the early stages were characteristic and usually diffuse. However, in a few cases only a portion of the biopsy showed changes suggesting that it was possible for considerable variation in intensity of reaction in different portions of the liver to occur and that liver biopsies may not always give an accurate picture of the changes present. This in part may account for the well known observation that there is frequent discrepancy between the histologic changes and the clinical and laboratory findings.

Microscopically, in the more severely involved specimens, the liver architecture appeared distorted under low power. The variation in the size of liver cells, the variation in tinctorial reaction, and the infiltrate, more prominent in the portal areas but sometimes spreading throughout the section, accentuated the apparent alteration in architecture. Under higher power, isolated cells and small groups of cells were swollen and distorted. The cytoplasm was deeply stained with eosin and appeared cooked and smudgy. Nuclei were very large, were sometimes hyperchromatic and sometimes vesiculated. Occasional mitotic figures were present. Progression of these changes to the "Mallory Body" stage could be seen. Although these changes were most frequently found about the central vein, they could also be found anywhere in the lobule.

Another type of cellular change affected isolated cells. These were considerably enlarged. The cytoplasm was vacuolated and cell borders were indistinct. Such isolated cells appeared to undergo necrosis by lysis.

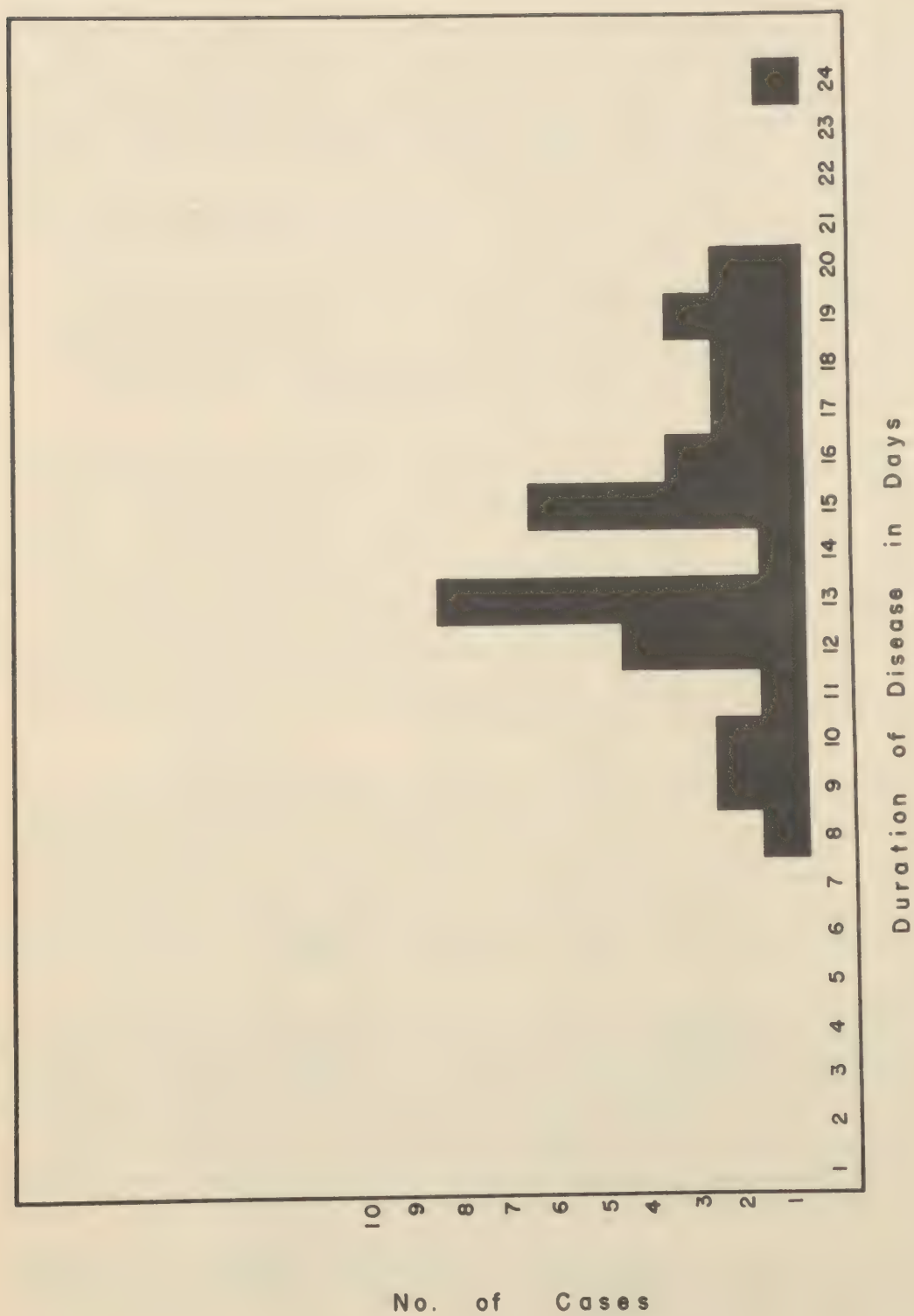Duration of Disease in Days

No. of Cases

Figure 2. Duration of Disease at Time of First Liver Biopsy

Although mononuclear cells were most numerous in the cellular infiltrate, eosinophiles and sometimes polymorphonuclear leukocytes were often present. Kupffer cells were moderately prominent.

The second series of biopsies taken 28 days after the first biopsy, with some exceptions, showed considerable resolution and evidence of healing although in no case was there yet a complete return to normal. Generally the architecture appeared normal. Occasionally a small condensed eosinophilic "Mallory Body" was seen. Liver cells sometimes contained two nuclei. The inflammatory infiltrate was restricted to the portal areas and to small foci within the lobule. The latter suggested accumulation about single necrotic liver cells and is described by Dr. Smetana at the Armed Forces Institute of Pathology as hepatitis with focalization. There were 8 cases which did not show this degree of healing, although 3 showed considerable healing when compared to the original biopsy.

Several biopsies of the pretreatment series showed condensation of fibrous or reticulum tissue in the periphery of some lobules. Although at first sight this appeared to represent fibrosis and scarring, special stains including the Masson trichrome stain and the Wilder reticulum strain showed that the widened infiltrated portal areas seen in many biopsies did not represent scarring but portal edema due to inflammation and some collapse of stroma. Only three biopsies of the pretreatment series showed minimal fibrosis. Approximately half of the repeat biopsies showed by special stains definite portal changes of a minimal to slight degree. Although these were described as the changes of postnecrotic scarring it is suspected that such scarring could contract to such a degree that it would not be apparent after complete healing. In this group there was no evidence of scarring which could go on to cirrhosis.

One other change found in the repeat series of biopsies consisted of minimal to mild fatty metamorphosis in 23 cases. Although this was never severe it represented a definite change in the two series, since none of the first series of biopsies showed any fatty metamorphosis. Similar fatty change had been noted in 6 of 84 biopsies in the investigation carried out during the first part of the year. There was no relation between the presence or degree of fatty metamorphosis and treatment with vitamin B_{12}. However, all patients were on a high caloric, high protein diet and many gained a great deal of weight. It is suspected that the fatty metamorphosis reflected this weight gain and had no other significance.

All but five patients showed evidence of healing when the two biopsies were compared. One of these 5 patients was first biopsied on the 19th day of illness when his symptoms were moderately severe (TSB 13.6). Biopsy showed moderate hepatitis with no change 28 days later although his clinical findings had improved considerably (TSB 1.5). The other four cases had moderate hepatitis, clinically and histologically, at the time of the first biopsy 8, 13, 15 and 16 days respectively after onset of illness. They had improved considerably by clinical standards at the time of the second biopsy although the latter showed little evidence of healing. Even in these 5 cases there was no evidence of progression of the histological changes.

It is believed that the damage to liver cells which occurs in infectious hepatitis takes place during the first week or less of the disease in most cases, that thereafter cells degenerate, recover or regenerate in proportion to the degree that they are affected, that healing in this type of injury is a relatively slow process, that most of the prolonged duration of hepatitis is related to this healing phase, and that in the usual non-fatal form of infectious hepatitis, individual cells and small clusters of cells are affected rather than large units such as lobules. The variation in severity of the clinical disease therefore would be related to the number of cells damaged. It is realized that cells showing no morphologic changes may be affected physiologically and that this is more likely when there is extensive histological changes. On the other hand, in later stages of the disease cells still showing morphologic changes may be in a healing stage and the hepatotoxic action of the virus may have been neutralized or dissipated. The morphologically unaltered cells may then have recovered normal physiologic functions. This would explain the five cases described above which showed persistence of histologic changes with clinical recovery. Unfortunately, we have not learned how to recognize such a status on the basis of histologic changes alone.

PRISONERS OF WAR: During the last 4 months of 1950, following the Pusan perimeter "Break Through", large numbers of Communist troops were captured. Most of these were North Koreans. Subsequently numbers of Chinese Communists were also captured. About the end of 1950 the prisoners were brought together in large camps in the Pusan area.

From the beginning, infectious disease of all types were extremely prevalent among these prisoners. In January of 1951 enteric infections had reached true epidemic proportions. Medical and hospital facilities were taxed beyond capacity. The situation was aggravated by the large number of surgical complications attending salmonella infections. When the enteric infection epidemic was controlled, tuberculosis became the important reason for hospitalization. Throughout, parasitic infestation, relapsing fever, and malnutrition were constant problems.

Because of the epidemic and the attendant medical load, the language difficulties, and the differences in cultural and sanitary approaches to disease, clinical histories were difficult to obtain, were usually superficial and scanty, and were unreliable. Follow up was impossible. However, a large number of valuable surgical and autopsy specimens were obtained at considerable sacrifice of time and effort on the part of the medical officers at the POW hospitals.

Enteric Infections - Sanitary precautions were almost non-existent among the POW's and enteric pathogens present in the prisoner population were widely introduced and distributed. As a result, the epidemic was not due to a single organism but instead almost every known type of Salmonella and Shigella was recovered. Certain types predominated and were associated with complications in an unusually high percentage. Among the Salmonella, S. paratyphi and S. enteriditis were most frequently isolated while among the Shigella, Sh. flexneri 3a (Z) and Sh. flexneri 4a (103) were most frequently isolated. In addition there were numerous cases of amebiasis. Double and even triple infections were observed. Detailed clinical and bacteriological studies of this epidemic can be found in the Bacteriology Section and in the report of the Joint Dysentery Research Unit of the Enteric Disease Commission.

Salmonella with Intestinal Perforations - Early in the epidemic, before the etiology was recognized, there were a large number of spontaneous perforations of the ileum. Often at operation ascarids were found free in the peritoneal cavity but it was soon realized that they had no causal significance. Inspection of resected segments of ileum showed numerous oval narrow ulcers running parallel to the rugae which overhung the ulcers tending to hide them. Some of the ulcers were superficial, others extended to the peritoneum. In some cases there were single perforations, in others there were multiple perforations. In most cases there was a diffuse fibrino-purulent peritonitis. The mesenteric lymph nodes were enlarged and frequently necrotic. The possibility of a foreign body etiology, particularly ground glass, was investigated but was discarded when review of the literature revealed that even large amounts of ground glass caused no harm, and when glass fragments were identified in control cases as well as in resected portions of ileum. Approximately 250 patients were operated upon for spontaneous perforations. This laboratory received portions of intestine from 132 of these cases. It is not known how many unexplored patients died of peritonitis following such perforations. However, in 11 autopsies, intestinal perforations and peritonitis were found.

As mentioned above, the clinical history was not reliable and therefore, the following must be interpreted cautiously. The most frequent symptoms recorded were diarrhea, abdominal pain, anorexia, fever, and chills (Table XXXV). Most of the patients reported that the acute exacerbations of symptoms, interpreted to indicate the time of intestinal perforation, occurred on the first day of illness. However, many gave intervals up to 10 days (Table XXXVI).

The interval between the suspected time of perforation and of operation is given in Table XXXVII which would suggest that many of these patients had a considerable delay between the time of perforation and operation. The character of the peritoneal exudate

Table XXXV. Prodromal Symptoms In Patients With Perforating
Salmonella Infections

Symptom	No. of Patients
Diarrhea	29
Abdominal Pain	28
Anorexia	17
Fever	11
Chills	7
Malaise	3
Abdominal discomfort	2
Bloody stools	2
"Dysentery"	2
Headache	2

Table XXXVI. Interval From Onset Of Disease To Symptoms of Perforation

Interval	No. of Patients
Same Day	48
1 - 5 Days	19
6 - 10 Days	19
11 - 15 Days	7
16 - 20 Days	0
21 - 25 Days	1
26 - 30 Days	1
2 months	3

supports this finding in many cases. However, the histories of intervals of more than
5 days probably were not accurate. One patient had 3 separate perforations, 7 patients
had 2 perforations. The location of these perforations of the ileum in relation to the
ileocecal valve is given in Table XXXVIII. It is evident that most perforations occurred

Table XXXVIII. Location Of Site Of Perforation Of Ileum in Centi-
meters From Ileocecal Valve

Distance	No. of Cases (Single Perforations)
6 - 10	2
11 - 15	11
16 - 20	8
21 - 25	24
26 - 30	15
31 - 35	4
36 - 40	6
41 - 45	5
56 - 60	6
80	1

in the distal 30 cm. of the ileum, although some occurred even in the jejunum. Although
in 44 cases there was only a single ulceration in the resected portion of intestine, and
this the one which had perforated, as many as ten distinct ulcerations were found in
other cases (Table XXXIX). The length of the resected portions of intestine is given
in Table XL. The longest single portion of resected ileum measured 170 cm. The median
length of resected intestine was 31 to 40 cm. Cultures were made directly from ulcers in
76 cases. Positive cultures were obtained from the ulcerations in 20 cases, and negative

Table XXXIX. Number Of Ulcerations In Resected Portions of Ileum

No. of Ulcerations	No. of Cases
1	44
2	21
3	16
4	11
5	7
6	4
7	2
8	2
9	2
10	2

Table XL. Length Of Ileum Removed At Operation

Length in Cm.	No. of Cases
1 - 10	8
11 - 20	14
21 - 30	18
31 - 40	23
41 - 50	14
51 - 60	9
61 - 70	4
71 - 80	8
81 - 90	1
91 - 170	3

cultures in 56 cases. Of the 20 positive cultures, 18 contained S. paratyphi, 1 showed S. Schottmuelleri and 1 S. typhosa. Cultures were performed in all 11 autopsy cases. S. paratyphi was recovered in 3, S. typhosa in 1 and 7 were negative for enteric pathogens.

The gross appearance of the ulcers was described above. Both at surgery and in the autopsies of the fatal cases no consistent gross alterations were present in the liver and spleen.

Microscopically, the intestinal lesions showed a definite and consistent pattern, with considerable variation depending on the age and extent of the lesion. From the beginning this pattern appeared unusual in that there seemed to be no predilection for Peyer's patches, although there was unquestionable involvement of lymphoid tissue. The uninvolved portions showed marked edema of the submucosa and a mild diffuse mononuclear infiltration of the mucosa. As the ulcers were approached small lymphoid aggregates appeared in the mucosa which became progressively larger. These aggregates developed into typical well-formed follicles with active germinal centers, pierced the muscularis mucosa, and were found extending from the mucosa to the muscularis. Evidence of reaction became progressively severe as the lymphoid follicles increased in size and the larger ones contained small foci of necrosis. At this state small superficial areas of necrosis appeared in the mucosa. In the next stage of development, the necrosis within the follicles and in the mucosa appeared to join and an ulcer was found extending into the lymphoid tissue. With loss of necrotic material, the ulcers appeared to collapse so that they appeared very narrow and frequently were found between rugal folds. Larger ulcerations extended down to the muscularis and were surrounded by an intense inflammatory reaction in which mononuclear cells of all types including many phagocytes and plasma cells predominated. In many such ulcerations polymorphonuclear leukocytes and eosinophiles were often found and occasionally foreign body giant cells of the Langhan's type were seen. Frequently lesions at this stage

extended laterally resulting in a flask shaped ulcer with overhanging margins. The large swollen macrophages frequently present raised the possibility of a concomitant amebic infection. Special stains were used but in no case could amebae be recognized. The next stage was extension of the exudate into the muscularis with disruption of the muscularis and necrosis of the muscle bundles. The final stage was the actual perforation, the walls of which were necrotic and contained an inflammatory exudate which appeared to extend up from the serosa into the base of the perforation. In a few cases the necrosis was of a caseous type and, when Langhans giant cells were present, suggested tuberculosis. Tubercle bacilli were not found in these cases. Necrotic foci were present in most lymph nodes submitted. In a few, the necrosis appeared caseous and confluent. In the autopsy cases, necrotic foci were sometimes seen in the spleen and liver, without lobular localization in the latter. In the autopsy case in which S. typhosa was recovered, there were large, distinct mononuclear cell aggregates. These were not found in 3 autopsies in which S. paratyphi were recovered or in the 7 in which no specific organisms were demonstrated.

These lesions in the intestine appeared unusual both grossly and microscopically. The lack of Peyer patch involvement, the circular distribution, and the apparent progressive involvement of small lymphoid aggregates suggested a pathogenesis and progress different from that usually found in typhoid fever. The bacteriologic evidence and the large number of spontaneous perforations support the belief that in an epidemic such as this in which S. paratyphi predominated, there was a slightly but definitely different order of events from that usually seen in typhoid fever. The clinical observation that no cases of spontaneous intestinal hemorrhage was reported during the epidemic also supported this.

Salmonella Without Intestinal Perforation - There were 20 autopsies on patients who died of a Salmonella infection and who did not have intestinal perforation. Table XLI lists the organisms recovered.

Table XLI. Salmonella Recovered At Autopsy in 20 Cases

Organism	No. of Cases
S. enteritidis	10
S. paratyphi	4
S. cholerasuis	3
S. hirschfeldii	2
S. typhosa and S. oranienburg	1

Half the cases were caused by S. enteritidis and most of these deaths occurred after the epidemic had abated considerably. Three of these patients, all of whom died in June, had a concomitant Borrelia infection, two of whom apparently were cured of the relapsing fever but died of the salmonellosis while the third had evidence of both at autopsy. The relation between S. enteritidis infection and relapsing fever is discussed under the latter heading. All of the patients with S. cholerasuis, S. hirschfeldii, S. typhosa and S. oranienburg, and 9 of the 10 with S. enteritidis infections had a septic course. One patient with S. enteritidis infection had an enterocolitis. Two of the 4 patients with S. paratyphi had a terminal complicating Shigella infection which was believed to be an important factor in the fatal outcome while the other two had a typhoidal course. Table XLII lists the clinical course, the important autopsy findings, and the sites yielding positive cultures. Six patients had pneumonia, 4 had bacterial endocarditis and 4 had meningitis as complications of their infections.

The epidemic of salmonellosis in the POW camp was unusual because of the types of organisms recovered. Both S. paratyphi and S. enteritidis are infrequently encountered in the United States and rarely are the cause of human fatalities. In this epidemic, S. paratyphi caused the majority of infections (Table XLIII) and was associated with a high incidence of spontaneous intestinal perforation with almost no recorded cases of intestinal hemorrhages. In its uncomplicated form patients followed a typical

Table XLII. Findings In 20 Fatal Cases Of Salmonellosis

Sites of Culture

Clinical Course	Organism	Principal Autopsy Finding	Blood	Urine	Bile	Ulcers	Feces	Brain	Heart Veg.	Lung	Spleen	Lymph Node	Bone Marrow	Empyema Fluid
Septicemia	S. enteritidis	Bacterial endocarditis	+	+	+		+	+	+	+	+	+		
Septicemia Tetanus	S. enteritidis	Pneumonia	+		+		−	+		+	+	+		
Septicemia	S. enteritidis	Relapsing Fever	+		+						+			
Septicemia	S. enteritidis	Endocarditis, Relapsing Fever	+				−			−	+			
Septicemia	S. enteritidis	Meningitis	−	+	−		+	+			+			
Septicemia	S. enteritidis	Jaundice	+	+	+		+				+			
Enterocolitis	S. enteritidis	Subdural, intestinal, and renal hemorrhage	−		−		−				−			
Terminal septicemia	S. enteritidis	Generalized Tuberculosis	+					+			−			
Septicemia	S. enteritidis	Meningitis	−	−	−		−	−			−			
Septicemia	S. enteritidis	Relapsing Fever	+	+	+		+	+		+	+	+		+
Septicemia	S. cholerasuis	Pneumonia and Empyema	−		−		+				+			
Septicemia	S. cholerasuis	Meningitis and Jaundice	+	+	+		+			+	+			
Septicemia	S. cholerasuis	Pneumonia and Jaundice	−		−		+				+			
Septicemia	S. hirschfeldii	Pneumonia and lung abscess	+	+	+		−			+	+			
Septicemia	S. hirschfeldii	Bacterial endocarditis			−	+	+				+			
Septicemia (a) (b)	S. typhosa (a) S. oranienburg (b)	Bacterial Endocarditis	(a) − (b) +		+	+	+	+	+		+	− −	−	
Typhoidal	S. paratyphi	Pneumonia and abscesses		−	−	−	−	−	−	−	−			
Typhoidal	S. paratyphi	Streptococcus meningitis			+	+	+	+		−	+			
Typhoidal	S. paratyphi	Jaundice and Shigellosis		−	−	−	+	−		−	−		−	
Typhoidal	S. paratyphi	Pneumonia and Shigellosis	+	+			+				+	+		

Key: + = positive culture
    − = negative culture

typhoidal course, yet the study of the intestinal lesions indicated a progression of events which differed somewhat from typhoid fever. There were no cases submitted which showed a septicemic course or evidence of localization of the infection outside the gastrointestinal tract.

Table XLIII. Incident Of Different Types of Salmonella Recovered
in a Group of 203 Patients*

Organism	No. Isolated*	% of Positive Isolations
S. paratyphi	128	59.3
S. typhosa	21	9.7
S. cholerasuis	20	9.3
S. enteritidis	14	6.5
S. oranienburg	8	3.7
S. hirschfeldii	2	0.9
Others	23	10.6
	216	

* Double salmonella infections were present
in 13 of these patients

S. enteritidis was responsible for only a small percentage of the infections during the epidemic, but the organism continued to be recovered from patients throughout the year. Although the autopsies studied do not represent an accurate sample of the deaths due to Salmonella, it is probably true that infection by S. enteritidis was a greater threat to life than infection by S. paratyphi. During the epidemic only 7% of a group of 203 patients (Table XLIII) were positive for S. enteritidis but during this time this organism was recovered from almost as many autopsies as was S. paratyphi.

In the one case of double infection, the patient had an S. typhosa endocarditis and meningitis and yielded S. oranienburg from 2 ileal ulcers and from the urine. Generally, S. typhosa and S. paratyphi were not recovered as easily and from as many sites as were the other organisms.

There is one other feature which cannot be evaluated, but which could be of importance in explaining some of the unusual features of this epidemic. Many of the prisoners showed evidence of chronic malnutrition which did not respond to dietary treatment. This was even more prominent in the patients who died of bacillary dysentery and in both groups may have so reduced resistance that infections would be more severe and complications more likely.

Dysentery - Thirty-five patients died of dysentery. Of these, 26 prisoners died of the bacillary form, 8 of the amebic type, and 1 person had both amebic and bacillary dysentery. Shigella organisms were cultured in ten of these patients while in the remainder, the clinical course and autopsy findings were highly suggestive.

Shigellosis - Although Shigellosis was extremely prevalent among the prisoners of war during the epidemic, uncomplicated bacillary dysentery was seldom a fatal disease. A peculiar sequence of events occurred among these fatal forms. These patients were hospitalized because of bloody diarrhea and dehydration. Sulfadiazine therapy had little effect on the course of the diarrhea and these patients gradually developed edema culminating in extensive anasarca. Peculiarly, the edema and anasarca developed even in a few instances in which the diarrhea was halted and controlled by sulfadiazine.

The usual feature at autopsy in these cases was an acute colitis. The mucosa was usually edematous, hyperemic, showed small hemorrhages and was often covered by a syrupy brownish-red fluid. Microscopically, a diffuse polymorphonuclear cell infiltration occurred in the mucosa and within a fibrinous exudate that occasionally covered

the surface. Edema of the submucosa was often marked, but the muscular coat and serosa were uninvolved. In one unusual case, two different Shigella flexneri organisms were isolated - one, from the colon (Sh. flexneri 4), the other, from granulomatous mesenteric lymph nodes and spleen (Sh. flexneri 3). The granulomatous lesions contained areas of caseation, epithelioid cells, and Langhans giant cells, but both special stains and guinea pig inoculation failed to demonstrate acid-fast bacilli. Three features of this case were unusual: (1) the double Shigella infection, (2) the dissemination of Shigella to lymph nodes and spleen, and (3) the suggestion that Shigella may cause a granulomatous lymphadenitis.

Amebiasis - Amebiasis was the cause of death in 6 cases. Autopsy in 4 cases showed only severe colitis. In one case, only a liver abscess was found. In the sixth case there was amebic colitis and a liver abscess which had perforated and formed a subdiaphragmatic abscess. Surgical drainage was instituted but an extension of the infection through the diaphragm into the pericardial cavity occurred and the patient died of amebic pericarditis.

Exfoliative Cytology - In June 1951, it was determined to reinvestigate the types of exudates present in bacillary and amebic dysentery and to attempt to apply newer methods to a study of exfoliated intestinal cells in such exudates.

At the time of proctoscopic examination of patients with acute dysentery a culture was taken and material obtained by pipette aspiration directly from ulcerated or inflamed mucosa was used to make smears. In order to make thin smears, 0.75% saline was used to dilute this material. The stool specimen passed after proctoscopy was diluted with 0.75% saline filtered through cotton and a portion of the supernatant fluid was used for smears. The material for smear was placed on a glass slide, and then spread as for a blood smear. This was allowed to dry in air and was then fixed with methyl alcohol. Poly-vinyl alcohol fixation proved to be unsatisfactory and was discontinued after a short trial. Giemsa stain was used routinely. Subsequently, the results of cultures and examinations for ameba were compared with the results obtained by studying the smears. Table XLIV gives pertinent data concerning these cases.

Table XLIV. Dysentery Series

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Amebic dysentery cases ...............  56
        Proctoscopic smears ........  34
        Stool smears ...............  22

Bacillary dysentery cases ...........  68
        Proctoscopic smears  .......  35
        Stool smears ...............  33

Acute dysentery etiology unproven ... 181
        Proctoscopic smears ........ 113
        Stool smears ...............  68
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In 181 cases (59.3%) a specific cause of the dysentery was not demonstrated. In the remaining cases smears from patients with amebic dysentery more frequently showed macrophages and mononuclear cells and only a few slides showed many polymorphonuclear leukocytes. Smears from patients with bacillary dysentery had a significantly higher percentage of polymorphonuclear cells and only few mononuclear cells. This, of course, has been described in the literature. However, it was not believed that a diagnosis of either bacillary or amebic dysentery could be made on the basis of smears alone although they could be used as an indicator of the probable type of infection during an outbreak of dysentery. Obviously cultures and wet preparations would have to be examined to establish the diagnosis.

No significant observation was made with the method used in the study of exfoliated cells of a characteristic or unusual reaction either for diagnostic purposes or to demonstrate the pathologic processes occurring in the intestinal mucosa. Exfoliated cells were recognized as intestinal mucosal cells in various stages of preservation

and degeneration. Smears made from aspirated material at the time of proctoscopy were far more satisfactory and much easier to interpret than those made from the stool specimens.

Parasitic Infestation - Intestinal parasites were found very frequently in POW's and the majority harbored more than one parasite. This had been demonstrated repeatedly by stool surveys. However, at autopsy with certain exceptions there was little evidence that the parasites had contributed to the patient's death.

A single examination of intestinal contents in a series of 60 autopsies on POW's using a concentration method satisfactory only for ova of helminths revealed parasites in 86.6%. In most cases more than one parasite was present. Table XLV gives the number of multiple infections and Table XLVI lists the parasites recovered.

Table XLV. Survey of Intestinal Contents in 60 Autopsies

No. of Parasites recovered (ova)	No. of Cases
6	1
5	2
4	6
3	15
2	15
1	13
0	8

Table XLVI. Parasites Found at Autopsy in 52 Positive Cases

Parasites		No. of Cases
T. trichiura		43
A. lumbricoides		43
Ova	32	
Adult worm - no ova	11	
Hookworm sp.		31
P. westermani		6
C. sinensis		5
E. coli		4
Trichostrongylus sp.		2
M. yokogawi		2
D. latum		1
E. nana		1

Ascariasis - Although this parasite was found with great frequency in the POW's, it ordinarily caused no symptoms or pathological changes. However, in two instances adult worms were found in the common bile duct causing partial obstruction. In another case, an adult worm was found in the liver. In all 3 cases there was an acute cholangitis and multiple hepatic abscesses. In two of the cases, bile thrombi were demonstrated in the pulmonary vascular bed. Foreign body granulomata were present about ascaris ova in the liver and lung of one patient and in the liver alone in another patient.

Paragonimiasis - Although infestation with this parasite frequently causes little reaction, in rare occasions the parasite establishes itself in situations other than the lung and may then cause a serious illness. One surgical and four autopsy specimens showed the presence of paragonimiasis. The surgical specimen was a portion of a brain abscess, the etiology of which was recognized at surgery. Another patient had signs and symptoms of a brain tumor. Autopsy revealed a brain

abscess containing N. catarrhalis superimposed on a large granulomatous reaction to paragonimus eggs. Lesions were also found in the lung and an abdominal lymph node. Death in the other cases was due to a widespread tuberculosis, an amebic and bacillary dysentery, and to a sensitivity reaction to pontocaine. In the patient who had tuberculosis, only pulmonary parasitic lesions were found. In the dysentery case, the parasites and ova were found in the lung, liver, seminal vesicles, spermatic cord, mesentery, and omentum. In the last case, typical granulomatous and cystic lesions containing parasites and ova were found throughout the lung and abdominal cavity. One such lesion at the right ureteropelvic junction had caused a slight obstruction and a mild hydronephrosis.

Clonorchis sinensis - During the year 1951, six cases of clonorchiasis were diagnosed at autopsy. All were in POW's - 5 North Koreans and 1 "Oriental". The age distribution was from 21 years to 41 years. All cases were in males. Primary causes of death were: carcinoma (2 cases), malnutrition (2 cases), perforated peptic ulcer (1 case), and salmonella septicemia (1 case). In 3 cases the pancreatic ducts were infested as well as the bile ducts. The liver weights ranged from 1,245 grams to 2,200 grams. The hepatic lesions caused by the parasites were biliary duct fibrosis, proliferation, dilation and peri-ductile inflammatory reaction. No definite evidence of portal obstruction was noted in any case. Collateral circulation was not observed, and splenomegaly was found in only one case. The weights of the spleens varied from 100 to 150 grams in 5 cases. The other case, one of salmonella septicemia, had a spleen typical of salmonellosis, weighing 375 grams. From this evidence it is concluded that significant portal hypertension was not present in any of these cases.

The functional capacity of the liver was not as easy to assess, since the degree of anatomical change was often not correlated with functional alteration. Both patients who died of malnutrition had jaundice and it is possible that clonorchiasis played a contributory role.

Miscellaneous - Virus and rickettsial diseases were infrequent among the POW's. No cases of epidemic hemorrhagic fever were recognized. There were 5 deaths due to infectious hepatitis, one due to smallpox and one probably due to typhus.

Tuberculosis was frequently present and was considered to be the cause of death in 40 cases. The distribution and findings showed nothing unusual.

Relapsing Fever - During 1951 the Pathology Department received material from 7 fatal cases of relapsing fever, 6 of whom were POW's and one an American soldier. A review of the 1,542 autopsies on file at this laboratory studied prior to 1951 revealed only one additional case. This patient was a 23 year old white soldier stationed in Korea who died on 27 December 1948 of a ruptured spleen. This case is included in the following discussion of the pathology of relapsing fever.

During 1951 relapsing fever was reported in 107 POW's and 69 United Nations troops, (Table XLVII).

Table XLVII. Reported Cases Of Relapsing Fever In Korea - 1951

	U.N. Troops	P.O.W.	Total
January	2	0	2
February	0	0	0
March	0	30	30
April	1	10	11
May	14	10	24
June	19	36	55
July	13	12	25
August	2	2	12
September	0	4	4
October	4	1	5
November	4	2	6
December	1	0	1
Total	69	107	176

The diagnosis was based on the presence of Borrelia in peripheral blood smears and on typical clinical findings.

A review of the available literature showed that there were almost no reports of fatal cases occurring in the U.S. and that relapsing fever was endemic in Korea. Fudlow referred to a total of 300 diagnosed cases at the Severance Union Medical College, Korea, during the 20 year period 1913-1933. In outbreaks in China, a peculiar relation between relapsing fever and S. enteritidis infection has been reported to occur with high frequency. One investigator demonstrated both spirochaetes and S. enteritidis in lice on these patients. Although three of the POW's who died had both Salmonella infection and relapsing fever, Salmonella infections were present in epidemic proportions in the POW camps. Relapsing fever and salmonellosis in these 3 patients therefore could have been coincidental.

All patients were males under 30 years of age except for one who was 47. Two were American soldiers (1 white, 1 negro), 5 were North Koreans and one was Chinese. The most frequent presenting complaints were sudden onset of malaise, chills, fever, and anorexia. The 3 patients with S. enteritidis infections did not have a relapsing course and were the only ones to complain of diarrhea. Symptoms had been present for 3 to 14 days prior to hospitalization at which time 4 patients had marked jaundice and one had mild jaundice. Three of the 4 patients who had marked jaundice had salmonellosis. The livers of 6 patients and the spleens of 4 patients were palpable and enlarged. Two patients with Salmonella infection had cutaneous ecchymoses. Petechiae, red blood cells in the spinal fluid, and a macular rash were described in one patient each who did not have such an infection. Central nervous system symptoms were frequently found. Six of the patients had stupor, clouded sensorium, delirium, disorientation or coma and 3 had meningismus.

In the fatal cases, antemortem diagnosis was made in 4 instances on the basis of Borrelia present in large numbers in peripheral blood smears. Spirochaetes were not observed in such smears in a 5th patient while the other 3 patients did not have blood smear examinations. White blood cell counts in 3 patients were 16,600, 25,000, and 58,000 respectively. Urinalysis usually showed moderate albuminuria and occasional to many red blood cells. A serologic test for syphilis was performed in only one case and this was two plus positive.

Four patients, including both American soldiers, died during the first hospital day and two died within three days of hospitalization. One patient died of salmonella septicemia three days after entering the hospital and one patient died of salmonella endocarditis 20 days after entering the hospital. Both of these patients apparently responded to treatment for relapsing fever since Borrelia could not be demonstrated after this therapy in either blood smears or tissue sections. A total of 5 of the 8 patients received mapharsen, penicillin or chloramphenicol alone or in combination as treatment for relapsing fever. Jaundice, concomitant Salmonella infection, and the central nervous system signs tended to confuse the diagnosis. Table XLVIII gives the diagnoses considered in these cases. Table XLIX indicates the fluids and tissues in which Borrelia were found.

Pathology - Two patients, apparently cured of relapsing fever, died of Salmonella infections. The pathology found at autopsy in these 2 cases is described in the discussion of salmonellosis and is not included in the following discussion.

At autopsy three of the remaining patients were obviously jaundiced and three patients had some diffuse lymphadenopathy. One patient with a complicating Salmonella infection had a fine reddish-brown macular rash in the inner aspect of the thigs. Another patient whose course suggested infectious hepatitis had diffuse bronzing of the skin and roughened and deeply pigmented skin of the hands and feet suggesting a pellagrinous dermatitis. The peritoneum and pericardium were bile stained in 3 cases and the pleura and pericardium contained petechiae in 2 cases.

Heart weights varied from 300 to 325 grams and averaged 310 grams. Cardiac valves and vessels showed no changes. Microscopically 5 of the 6 cases showed a mild to

Table XLVIII. Diagnosis Made in 8 Cases of Relapsing Fever

Case No.	Admission Diagnosis	Final Clinical Diagnosis	Gross Autopsy Diagnosis	Final Autopsy Diagnosis
J-563	Prodrome of infectious hepatitis	Ruptured spleen of undetermined cause	Ruptured spleen due to relapsing fever	Ruptured spleen due to relapsing fever
J-1623	Encephalitis vs poliomyelitis	Encephalitis vs poliomyelitis	Hemorrhagic diathesis with pontine and sub-dural hemorrhages	Relapsing fever
J-1642	F.U.O.	Relapsing fever*	Relapsing fever	Relapsing fever
J-1659	F.U.O.	Possible typhoid with acute hepatic necrosis	Fulminant infec-tious hepatitis	Relapsing fever
J-1966	F.U.O.	Relapsing fever*	Salmonella septicemia	Salmonella** septicemia
J-1985	F.U.O.	Relapsing fever* acute yellow atrophy (poss. Herxheimer)	Salmonella endocarditis	Salmonella** endocarditis
J-1992	Infectious hepatitis	Relapsing fever*	Relapsing fever	Relapsing fever
J-2284	F.U.O.	Salmonella** Septicemia	Salmonella septicemia	Salmonella** septicemia, Relapsing fever

F.U.O. - Fever of undetermined origin
* Diagnosis based on positive blood smears
**Diagnosis based on cultures

moderate myocarditis characterized by a mononuclear and histocytic interstitial infil-trate. Borrelia were demonstrated in vessels, interstitium, and myocardial fibers in 2 cases. The degree of myocarditis, as graded histologically, was more intense in those hearts in which Borrelia were present.

The lungs presented non-specific changes consisting of varying degrees of con-gestion, edema and bronchopneumonia. Borrelia were demonstrated in alveolar capillar-ies and walls in 2 cases.

Splenomegaly was present in all cases. Weights of spleen in 5 cases varied from 300 to 600 grams, with an average of 425 grams. The spleen was described as enlarged in the sixth case. In the case of rupture of spleen, the organ weighed 310 grams and was completely enveloped by blood clot. On its anterior surface a large, irregular area of rupture involved the mid-portion and lower pole. The capsule could not be iden-tified in this area. The color of the spleen varied from deep purple to purple-red. In 3 cases the "follicles" were prominently enlarged, particularly peripherally, and were described as tan-grey to yellow. These areas correspond to the miliary abscesses seen microscopically. One prosector described these "follicles" as resembling minute foci of suppuration. The pulp was soft and mushy in the spleen which ruptured and in

Table XLIX. Fluids and Tissues Examined For _Borrelia_

	Blood (clinical)	Spinal fluid (clinical)	Blood (autopsy)	Treatment*	Heart	Lung	Spleen	Liver	Brain	Kidney	Pancreas	Adrenal	Thyroid	Lymph Node	Bone Marrow	Testes	Prostate	Intestine
	Smears				Warthin-Starry Stains, Tissues													
J-563	0	0	∤	no	-	-	∤	∤	0	∤	-	-	-	-	-	-	-	0
J-1623	0	0	0	no	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤
J-1642	∤	0	0	yes	-	-	-	-	∤	0	-	0	0	0	0	0	0	-
J-1659	0	0	0	no	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤	∤
J-1966	∤	0	-	yes	-	-	-	-	-	-	0	-	-	-	0	-	-	-
J-1985	∤	0	-	yes	-	-	-	-	-	-	0	0	0	-	0	-	-	-
J-1992	∤	∤	∤	yes	-	-	∤	-	∤	-	-	-	0	0	0	0	0	-
J-2284	-	0	0	no	-	-	∤	-	-	-	-	-	-	-	-	-	-	-

∤ Spirochetes present
- Spirochetes absent
O Not examined
* Mapharsen, penicillin and/or chloramphenicol

the spleen described as containing the minute foci of suppuration. In the remaining cases the parenchyma was firm. One case presented irregular 0.5 to 1.0 cm mottled, red-gray areas of infarction along its notched border.

Microscopically, the spleen showed mild to moderate congestive change and evidence of splenitis. In addition the spleen of 3 of the cases showed the characteristic lesions of relapsing fever. In these 3, there were miliary abscesses occupying follicles and adjacent pulp areas. These abscesses were composed predominantly of polymorphonuclear leukocytes and less frequently contained macrophages and fragmented nuclear debris. In a fourth case, multiple septic infarcts were present. These infarcts were sharply demarcated from the surrounding pulp. Centrally, there were fragmented karyorhectic nuclei and polymorphonuclear leukocytes. Silver stains revealed large numbers of _Borrelia_ in the peripheral portions of the abscesses and intervening pulp. The spirochetes were most prominent about the periphery of the lesions and occurred singly in clumps, and as fragments. In the more central portions of the lesion fragments of organisms were more commonly observed. Bacteria were not demonstrated in these miliary lesions. Giemsa stains failed to demonstrate _Borrelia_. In the case with splenic infarction, _Borrelia_ were demonstrated in the peripheral areas of the infarct only. The surrounding pulp and central portions of the lesion did not contain spirochetes. This was the only case in which bacteria were found and these were gram negative bacilli present in the central necrotic portion of the infarct. This case had a positive culture for _S. enteritidis_ from the spleen.

The liver weights varied from 1950 to 2350 grams, with an average of 2210 grams. Grossly the organ was pale and brown to yellow. Architectural pattern was obscured in 4 of the 6 cases. In one case the organ became green following formalin fixation, with accentuation of markings. _Borrelia_ were observed in sinusoids and cord cells in this case. In the ? livers in which _Borrelia_ were demonstrated, foci of necrosis in lobules and Kupffer cell proliferation were observed. Patients who had jaundice showed more intense focal necrosis but there was no evidence of bile stasis.

The combined kidney weights varied from 290 to 550 grams, with a median of 350 grams. The kidneys varied from pale yellow to gray-yellow. Spirochaetes were found in 3 cases. When present they were seen in vessels, glomeruli, interstitial tissue and tubules. Three cases showed evidence of a lower nephron nephrosis of mild to moderate

degree. Borrelia were demonstrated in only one of these 3 cases. Another of the 3 cases had a complicating salmonella septicemia.

The meninges were bile-stained in 1 case. In 2 cases there were massive sub-dural hemorrhages and associated pontine, medullary, and cerebral hemorrhages. In 2 other cases microscopic hemorrhages were observed in the floor of the fourth ventricle and in the cerebral cortex.

Borrelia were demonstrated in the central nervous system in 4 cases. In 3, Borrelia were seen in vessels, leptomeninges and at sites of hemorrhage. In the fourth case, spirochetes were present in an area of focal arachnoiditis only. In 1 of the 2 cases with massive cerebral hemorrhage, Borrelia were demonstrated only in cerebral tissues.

The pancreas, adrenal, prostate, testes, bladder, thyroid, bone marrow, and gastro-intestinal tract displayed no significant gross or microscopic changes other than petechial hemorrhages of the intestinal mucosa in 2 cases. However, Borrelia were demonstrated in all tissues in 2 cases (Table XLIX). Spirochetes were seen within vessels, interstitium, and parenchyma singly and as tangled clumps. There were no associated morphological changes or cellular response in these areas.

LIAISON WITH OTHER MEDICAL UNITS: Close liaison was maintained with other medical units in the Far East Command. The majority of the laboratory officers were personally known to the staff of the Pathology Department because they had been assigned at least for a short time to the 406th Medical General Laboratory. Material for CPC was sent at weekly intervals to 19 hospitals in Japan, Okinawa, and Korea. At close of the year when the pathology services of the 1st Medical Field Laboratory were expanded, direct contact with medical installations in Korea was discontinued and all work reports and CPC's were transmitted through that laboratory.

By the end of the year, the distribution of CPC material had become routine procedure. This material, consisting of a detailed abstract of the available history, a clinical and pathological discussion, a photostat of the autopsy protocol, microscopic slides, lantern slides and photographs when available were kept in package units and sent out at weekly intervals to the various hospitals. All the CPC's had previously been discussed at a conference held by the members of the Pathology Department. In addition CPC's were conducted by a member of the staff at weekly intervals at two of the hospitals in Tokyo. A surgical slide conference was also conducted at one of the Tokyo hospitals at weekly intervals.

During the spring and again in the fall the hospitals south of Tokyo were visited by a member of the pathology staff. Laboratory procedures were reviewed with laboratory officers and suggestions were made to improve the services rendered and available. In addition, the station hospital at Sendai was visited during the summer, and the station hospital at Sasebo during the early fall. In July a member of the staff discussed the pathology of JBE at a conference in Pusan, Korea. In December, the pathology of EHF was discussed at a theater conference in Tokyo. During November, Dr. Webb Haymaker, consultant and neuropathologist from the AFIP, gave a series of talks on the various phases of his specialty in Japan and Korea.

Additional histopathological study sets were made available by the AFIP during 1951. A list of these study sets was published in the Surgeon Circular Letter, GHQ and approximately 30 doctors in Japan, Korea, and the Philippines requested loan of these sets.

Medical officers were assigned to the Blood Bank from the Pathology Department during the year. In addition to duties with the collecting teams, a survey was made of installations and depots handling and using blood in Japan and Korea. Their findings are included in the Blood Bank report.

PUBLICATIONS: Because of the difficulty in obtaining satisfactory containers, one of the technicians investigated the use of plastic bags and found that one made of polyvinylchloride available on the local Japanese market, was a great improvement over the metal containers previously used. A description of this bag including specification and experience with its use was made the subject of a short report submitted through channels for publication in the Armed Forces Medical Bulletin. A recommendation was made that these bags be made available to laboratories for shipment of tissue specimens.

An article on hemangioblastoma of the cerebellum and medulla including two case reports, and another article describing experiences and pathological findings in the first series of liver biopsies at the Hepatitis Center was submitted for publication. At the request of General DeCoursey, an article on the pathology of EHF for publication in the Year Book of Pathology and Clinical Pathology was submitted; another more detailed article on the same subject was prepared for the symposium on EHF to be published in a journal so far unnamed. It is expected that the material for publication on the following subjects will be submitted in 1952:

Paragonimiasis including brain lesions; Liver biopsy during early stage of hepatitis; Enteric infections; Pathology of war wounds; Frostbite; Fat embolization in deaths due to trauma; Relapsing fever; Lower Nephron Nephrosis.

TRAINING ACTIVITIES: As in 1950, medical officers assigned to the 406th Medical General Laboratory were given an orientation course during which they spent time in all departments of the laboratory. Most of these medical officers had been on pathology residency training programs and their training was continued in the form of weekly CPC's, slide conferences and discussion of interesting cases. During 1951, one member of the department was certified by the American Board of Pathology. Enlisted personnel received continuous on-the-job training.

OFFICER PERSONNEL: During 1951, a total of 36 medical officers were assigned to the Pathology Department which was in effect a pool for medical officers with laboratory training. Of these officers, 12 were regular army, 3 were navy and 21 were reserve officers. Three of these officers were on continuous duty in the department during the entire year. Seven, including two navy doctors, remained in the department during their entire tour of less than a year in the theater, 8 returned to the Z.I., and 18 were assigned to hospitals in Japan and Korea following the period of orientation training described above. Of these 36 officers, one was a certified board pathologist and one was so certified during the year; another officer was certified after his return to the Z.I. At the end of the year there were 7 officers on duty in the Pathology Department.

During a period of 6 weeks an administrative MSC officer was assigned to the Pathology Department. He was of great assistance in organizing and administering non-professional activities of the department. Unfortunately, when the T/O was developed, it was not anticipated that a pathology department of a general laboratory would perform so many activities, and no provision was made for assignment of such an officer to the Pathology Department.

DIFFICULTIES ENCOUNTERED: No unusual difficulties other than those to be expected from the situation were encountered. As in 1950 the rapid turnover of medical officers was not considered a difficulty because of the advantages resulting from personal contact with officers who were to be assigned to other medical organizations in the theater.

RECOMMENDATIONS: As in 1950 it is again recommended that the "Standard Form 515" (Tissue Examination) be altered to provide space for recording the serial number of personnel of the armed forces. Also, it is again recommended that residency training programs for laboratory officers include a short course on army administration.

DEPARTMENT OF BACTERIOLOGY

As in previous years, in addition to the standard diagnostic support of medical installations, close liaison was maintained with other departments of the 406th Medical General Laboratory and with other laboratory units in the Far East Command. As a result the Bacteriology Department was directly concerned in the following three major problems: the bacteriology of war wounds, studies of enteric infections among prisoners of war, and the etiology of epidemic hemorrhagic fever. Some of the studies reported here were initiated during 1950, while others were started in 1951. Some problems were part of studies of which various phases are described in this report by other departments.

A total of 163,714 routine procedures were conducted in examining 45,812 specimens during 1951, representing an increase of 108% in procedures and 70% in specimens when compared with the work performed in 1950. Most of this increase was due primarily to the Korean War and the outbreak of enteric infections in the prisoner of war camps. A significant proportion of the work was devoted to processing and/or testing materials for intravenous therapy. This included the large scale preparation of convalescent serum and testing for the presence of pyrogens large quantities of fluids intended for intravenous use.

A statistical summary of the work performed is given in Table I below.

Table I. Statistical Summary of Bacteriology Procedures

Smears, Darkfields, and Microscopic Examinations 5,304
    GC, H. ducreyi, wounds, etc. 330
    Treponema pallidum and Borrelia sp. 120
    Leptospira sp. 1778
    Acid-fast bacilli 3076

Cultures 28,384
    Ear, nose, throat, etc. 619
    Blood fluids 772
    Urethra a/o cervical 571
    Wounds and tissues (aerobe and anaerobes) .. 1244
    Leptospira sp. 1489
    Autopsy 231
    Tubercle bacilli 6713
    Food, processed and canned 259
    Stools and rectal swabs 1329
    Sterility test 915
    Cultures submitted for identification:
        Possible Shigella and Salmonella sp. 12065
        Other organisms 772
    Cultures, miscellaneous sources 1405

Sanitary Bacteriology 46,888
    Milk and Dairy Products 2322
    Water, Ice, etc. 44566

Animal Inoculations 6,299
    Virulence and toxicity 26
    Pyrogen tests 940
    Clostridia inoculations 1020
    Leptospira inoculations 498
    Mouse virulence tests 210
    Guinea pigs for tuberculosis 3605

Table I. Statistical Summary of Bacteriology Procedures Continued

```
Diagnostic Antibody Determinations ..........................  10,438
    Febrile agglutinins (typhoid-paratyphoid etc.).....  7873
    Leptospira ........................................  2082
    Antistreptolysin ..................................   483

Other Diagnostic Procedures .................................   6,121
    Acrolein tests ....................................  3010
    Antibiotic assay (including tissues) ..............   204
    Sensitivity of organisms to antibiotics ..........  2146
    Guinea pig and hamster autopsies ..................   761

Biologics Production (Diagnostic) ...........................  43,345
    Autogenous vaccines ...............................   103
    Diagnostic antigens produced:
        Typhoid "O" and "H", cc. ......................  2613
        Paratyphi A, B, and C, cc. ....................  2618
        Proteus OX series, cc. ........................  8400
        Pasteurella sp. cc. ...........................  1130
        Brucella sp., cc. .............................  1250
    Diagnostic antisera produced:.....................
        Shigella sp., cc. .............................  1659
        Leptospira, Salmonella, other, cc. ...........  1550
        Absorbed single factor sera, cc. .............   307
    Human convalescent serum, EHF, cc. ............... 21000
    Rabbit serum, cc. .................................  2715

Diagnostic Antisera issued, cc. .............................   2,399
Diagnostic Antigens issued, cc. .............................  11,490

Cultures (new strains) lyophiled ............................   3,046
                                                             ────────
                                                              163,714
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TEST OF THERAPEUTIC FROST-BITE SOLUTION FOR PYROGENS: In anticipation of the occurrence of cases of frost-bite, 12,000 bottles of intravenous anti-frost bite solution consisting of glucose, alcohol, and procaine were ordered from Japanese manufacturers. The Bacteriology Department was assigned the task of testing all lots for the presence of pyrogens. Subsequently an order was placed for an additional 59,400 bottles of this solution. Because of space and personnel restrictions, modifications of the original procedures were employed to handle the larger volume to be tested.

U.S. Pharmacopeia (1) standards were followed except that a minimum of 1% of each batch of 1,200 bottles was tested rather than the usual minimum of 2%. This was considered acceptable because parallel tests were to be conducted by a civilian laboratory. A single test consisted of a pool of equal parts from each of 3 bottles injected into at least 3 rabbits. Since four such tests were performed for each batch of 1,200 bottles, 12 rabbits were used for each batch.

In the first series of tests, all glassware was cleansed in H_2SO_4 - dichromate solution, thoroughly rinsed in tap, distilled, and pyrogen-free water, dried, and heated in a muffle furnace for 30 minutes at 280°C. to insure freedom from pyrogens. Needles were similarly heated. Four temperature readings of rabbits were taken at 2 hours interval during one day, followed by single temperature readings for 3 days prior to initial test. The prescribed normal temperature limits of 38.9°C. to 39.8°C were observed, and rabbits with temperatures outside this range or which showed variation of over 0.6°C. during the day prior to test, were eliminated from use. In testing solutions, 10 ml. per kilo of animal weight was injected intravenously, at a rate not exceeding 10 ml. per minute, with the solution at 37°C. Temperatures were

taken one hour before injection, and at three hourly intervals after injection. Two or more rabbits with temperature rise of 0.6°C. constituted a positive pyrogen test while one such rise constituted a doubtful reaction requiring re-testing. Sterility tests were also carried out using 10 cc. of solution in 30 cc. of thioglycollate broth. Each bottle tested was cultured separately.

When the second lot was tested, the cleaning procedure was simplified. Glassware and needles were soaked for one hour in a detergent solution composed of 3 parts Na_3PO_4 and one part of $Na_4P_2O_7$. Thorough rinsing with distilled and pyrogen-free water, prompt oven drying, and immediate autoclaving gave completely neutral glassware which was pyrogen-free in over 100 individual tests.

The original herd of 48 rabbits used for testing the solutions was increased ultimately to 120. The batches of solution were increased to 1,500 bottles each and as many as 8 batches were tested in one week. Twenty-five lots of 1,500 bottles each were pyrogen tested and sterility checked through December 1951.

Although chemical tests for pyrogens have been proposed (2) the standard procedure still involves the injection of rabbits with the test substance and observation of the animals for temperature rises. These instructions state ..."Take four rectal temperature readings on each animal at 2 hour intervals 1 to 3 days before use ..... Do not use in the test those animals with a temperature in excess of 39.8°C." Beyond this statement no specific information was found on search of available literature regarding the range of temperature variation occurring in rabbits under conditions of this test. Some equivocal results were obtained during the early stages of this work, and it appeared that the handling of animals in the process of taking temperatures might be a factor in causing this temperature fluctuation. The following study to clarify this point was conducted. Three groups of 12 normal, healthy rabbits, weighing from 1,800 to 2,800 grams, which had been in stock for not less than 1 month were selected. Temperature in the animal room was maintained at 24° - 27°C., during the period of observation. During a test day the temperatures of the rabbits were taken at 2 hour intervals for a total of 12 hours observation. Temperatures of the rabbits of group I and II were taken on one day and this was repeated 3 days later, while those of group III were taken on 6 alternate days. The average temperature of all rabbits and the maximum dispersion from the mean were determined. Results are shown in Table II.

Table II. Temperature Variation in Normal Rabbits On Successive Observations

Group (12 rabbits)	Test Run	Ave. Temp. ° C.	Maximum dispersion From Mean In Degrees C.
I	1st	38.84	0.28
	2nd	38.66	0.23
II	1st	38.91	0.23
	2nd	39.33	0.10
III	1st	38.87	0.31
	2nd	38.91	0.30
	3rd	38.92	0.19
	4th	39.16	0.17
	5th	39.02	0.10
	6th	39.00	0.09

The variation in rabbit body temperature was affected by the number of times the temperature was taken. In group I, the range of dispersion was such that even on the second run, variation from the norm sufficient to constitute a positive reaction would be theoretically possible. It is to be emphasized that this fluctuation did not include any rabbits failing to qualify for inclusion as test animals by a temperature range as prescribed in the U.S. Pharmacopeia. Group II showed more uniform temperatures

and the range of variation in the second run was well within that permissible for a negative test. Group III showed a decrease in dispersion of temperature which accompanied increased numbers of observations.

On the basis of this experience it is apparent that the temperatures of the test rabbits should be taken repeatedly prior to performing pyrogen tests to avoid temperature fluctuations due to handling.

EVALUATION OF PENICILLIN SYRETTES: Assay of representative samples of 5,000 procaine penicillin syrettes (Squibb) was carried out in January 1951, to determine whether on the basis of supposed leakage, the syrettes were of rated potency. The stability of the preparation was also investigated.

The capacity of the syrettes was listed at 1.0 cc. and the potency at 300,000 units. The average weight of drug in the tubes was found to be 1.25 grams. The potency of the contents was assayed by a modified agar well technique (3). Twenty tubes sampled at random gave a mean value of 338,600 units per tube, with a mean deviation of 26,300. The syrettes were therefore of rated potency.

The effect of temperature on the penicillin was evaluated. Freezing (-10°C) did not affect the penicillin. After 48 hours at 37°C., an average loss of 10% in potency occurred in 5 samples; after 96 hours a loss of 25% to 35% of rated potency occurred in 10 samples. At 55°C., deterioration was still more rapid. There was a loss of 25% of rated potency after 48 hours and 70% after 96 hours in 10 syrettes.

The procaine penicillin was found to be non-homogeneous. Ninety percent of the penicillin was concentrated in the sedimented portion when the tubes were centrifuged.

PRODUCTION OF PROTEUS OX ANTIGEN: Difficulty has been frequently encountered in the preparation of standard suspensions of killed Proteus OX organisms. The strains are capricious and successive lots of antigen may show different agglutinating properties. The difficulty of maintaining supplies of positive human test serum adds to the difficulty of maintaining control of antigen sensitivity. Heat and phenol has been the method chiefly used in killing the organisms. During 1951 the alcohol treatment technique of Bien was tried with all 3 OX strains, and in the case of OX-19 and OX-2 very suitable preparations were obtained. However, repeated efforts failed to yield a salt-stable suspension of OX-K with this technique, and killing with heat and phenol was hence employed. The use of these antigens was complicated by the fact that suspensions became more agglutinable on standing. After several months of storage they could react to much higher titers than when freshly prepared. Several reports of OX-19 and OX-K titers above 1:640 were received in this laboratory during 1951. Levels of 1:20 to 1:40 were obtained when the same sera were tested here. The technique of antigen preparation was studied to find an explanation for these discrepancies. Two strains labeled 40-B-3 and "L" from AMS were used. Variations in biochemical reaction found in these strains are shown in Table III.

Table III. Cultural Variants Derived From Strains Of Proteus OX-K

Reaction and Time of Appearance

Strain and Colony	Maltose	Sucrose	Lactose	Xylose	Glycerol	Trehalose	Citrate	Indol	H2S	Gas
40-B-3-A	≠1*	≠1	-	≠1	-	≠3	≠	-	-	≠
40-B-3-B	≠3	≠1	≠1	-	≠7	-	-	-	-	-
40-B-3-C	≠5	-	-	≠1	≠7	≠1	-	-	≠	-
L	≠1	≠5	-	-	≠7	≠1	≠	-	≠	-

* Figure indicates day on which fermentation occurred

The variants shown were all non-motile. Colony morphology varied as follows: 40-B-3-A and 40-B-3-B colonies were 1.5 to 2 mm. in diameter with slightly irregular or rough edges; 40-B-3-C colonies were 1 mm. in diameter, and translucent, while L-D colonies were 1 mm. in diameter and opaque. Trial runs showed that 40-B-3-C, the translucent colony, yielded suspensions reacting to highest titer in control sera. This strain conformed most closely to the classical description of Proteus biochemical behavior, and was the most satisfactory source of Proteus OX-K antigen preparations.

The cultures were seeded to broth tubes from which agar slants were inoculated at 18 hours. Alcohol treated antigens were salt-unstable, but phenol-heat killed suspensions were stable and reacted on first trial to titer of 1:160 in scrub typhus convalescent serum. Production volumes of antigen, however, reacted to only one-fourth the titer of pilot preparations. When culture was seeded from dry agar slants and killed with 0.3% formalin in saline, an antigen was obtained which reacted to higher titers in control serums than when phenol-heat killing was used.

The following technique was used for the production of OX-K antigen:

1. Smooth translucent colonies containing non-motile organisms on subculture were selected.

2. Seed cultures were passed on dry agar slants.

3. Parallel seed cultures were tested for agglutinability prior to inoculation of flasks.

4. Kolle flasks of dried agar were inoculated from seed slants using a swab rather than a broth suspension.

5. Twenty-four hour growth was killed in 0.3% formol-saline at room temperature.

6. Stock cultures were maintained on agar slants and in lyophiled tubes.

EVALUATION OF SHIGELLA AND SALMONELLA ANTISERA: Because unpredictable deterioration may occur in diagnostic antisera, particularly with Shigella and Salmonella, a complete series of AMS Shigella typing sera was tested by cross-agglutination with homologous and heterologous strains. Among the results, Sh. flexneri 4 serum proved to react only with Sh. flexneri 5. Two Sh. boydii types did not react in Sh. boydii group antiserum, and Sh. dysenteriae group antiserum was deficient in antibodies for half of the types included in this species. A similar test of commercially prepared Shigella grouping serum showed some samples to be deficient in agglutinins for specific types in Sh. dysenteriae, Sh. flexneri, and Sh. boydii species. Salmonella typing sera, when assayed, revealed occasional lack in antibody, which in case of differentiation of Sal. enteritidis and Sal. blegdam caused four months delay in recognition of the latter type. Sera prepared by the 406th Medical General Laboratory were similarly tested and deficiencies in some types were at times noted, although these were less extensive.

CULTURAL DEMONSTRATION OF H. DUCREYI IN PENILE LESIONS: Since the description and isolation of Hemophilus ducreyi over 50 years ago, only sporadic attempts have been made to apply cultural techniques for the diagnosis of this disease. Difficulties have been so frequently encountered that to date there is no generally accepted diagnostic cultural procedure. Teague and Diebert (4) pointed out the intrinsic difficulties in identifying the organism in stained smears of exudate. When they used heat-inactivated clotted rabbit blood as a culture medium as suggested by Himmel in 1901 and by Fischer in 1903, they obtained morphologically positive mixed cultures in 140 of 274 cases of penile ulcer. Attempts to grow the organism on blood agar plates were reported in 1920 by Moore (5), who obtained positive cultures in only 5 of 55 cases. The cultural method was seldom successful during the next decade, and the view expressed in 1935 by Cole and Levin (6) that

"cultivation of the Ducrey bacillus is a most difficult procedure" was generally accepted. In 1938, Greenblatt and Sanderson (7) found that the organism could be grown with moderate success in whole blood. Using a pure culture they reproduced a typical textbook picture of clinical chancroid in a human volunteer, thus adding weight to the validity of the causative role of the organism. In 1945, Beeson and Heyman (8) used defibrinated rabbit blood for primary culture of genital lesions and obtained "positive" cultures in 42 of 50 cases. Sub-culture to 1% agar plates containing 5% rabbit blood yielded 19 isolates of H. ducreyi from these mixed cultures. They recommended defibrinated rabbit blood as a culture medium for clinical laboratory diagnosis.

Earlier comparisons (9) of penicillin, aureomycin, streptomycin, and chloromycetin in this laboratory showed that a stock strain of H. ducreyi was susceptible to such an extent that these substances could not be used in culture media to inhibit the usual concomitant flora. However, a slight growth stimulation with sub-inhibitory doses of chloromycetin was noted. This suggested that small amounts of antibiotic might serve to enhance recovery of the organism on blood agar. Levels above 0.5 units of penicillin, 0.3 gamma of streptomycin or 0.05 gamma of chloromycetin were found to inhibit growth of the organism. Chloromycetin and penicillin blood agar plates were then employed in attempts to isolate H. ducreyi in pure culture, in conjunction with a comparative evaluation of rabbit serum and serum broth in presumptive diagnostic demonstration of this pathogen.

Cultures were taken with a sterile platinum loop from beneath the edge of the penile lesions of a series of patients referred to the laboratory for examination for chancroid. At the same time the direct smears were prepared for comparison with culture, although no attempt to reach a diagnosis from such smears was made. The material for culture was inoculated into heat-inactivated clotted rabbit blood and into 50% rabbit serum tryptose phosphate broth. After preliminary trials, direct plate isolation attempts were omitted, since no significant results were obtained.

Cultures were incubated at 37°C. in a candle jar and examined daily or until positive findings were obtained, for a total of 3 days. The serum and serum-broth cultures were sub-cultured to fresh blood agar plates made with 20% human citrated blood and 1% yeast extract. Penicillin plates, (0.05 units/ml.) chloromycetin plates (0.05 gamma/ml.) and unaugmented plates were run in triplicate. These were also incubated in a candle jar to insure presence of 2.5% CO and a moist atmosphere, since these factors are considered to enhance growth of the organism.

The appearance of the organism in serum and in serum-broth was characteristic; long chains of coccobacilli appeared in pairs and multiple rows, tangled and twisted, and usually arranged in parallel "railroad track" pattern. Heavy contamination usually resulted in chains which were shorter and not distinctive. Distinction between H. ducreyi chains and those of decolorized streptococci may be readily made. The colonies on blood agar were translucent to gray, slightly dull, with concentric raised and depressed areas appearing on aging. Colonies were usually 1 mm. in diameter, and showed a tendency to remain intact when pushed across the agar surface. Although a slight hemolysis is referred to in the literature, we did not observe it in our cultures.

To avoid breaking up the chains of bacilli in making smears from liquid media, a sterile pipette was used to remove 0.05 ml.of culture, which was applied to a slide in a spiral pattern, to form a continuous film. The coiled "railroad tracks" of H. ducreyi persisted well with this technique. Blood agar sub-cultures, when obtained, showed diplobacilli and short chains of somewhat pleomorphic rods slightly thicker than those seen in the original broth. Table IV shows comparison of results of culture of 83 specimens in the two liquid media.

The persistence of the characteristic appearance in tryptose-phosphate serum broth is greater than in rabbit serum, which probably accounted for the greater number of positive smears. The two substrates were of approximately equal value in number of positive cultures obtained. The use of two media greatly improved the chances of diagnosis.

Table IV. Growth of H. ducreyi in Serum and Tryptose-serum Broth
Cultures of 83 Specimens

	Results Of Periodic Microscopic Examination		
Time	Positive in Tryptose-serum Only	Positive In Serum Only	Positive In Both Media
24 hr.	10	8	9
48 hr.	12	13	9
72 hr.	13	4	4
Total No. of cultures positive	15	13	18

A total of 46 positive cultures (55.4%) were obtained. Seventy-one percent of these positive cultures were recognized in tryptose serum, and 67% in rabbit serum. The interfering flora, chiefly Proteus and Pseudomonas spp., was deemed the main factor in failure to recover H. ducreyi from broth and serum mixed cultures.

All broth and serum cultures were plated at 24, 48, and 72 hours, on the solid media described above. Only 2 strains were recovered on the penicillin blood agar plate, one of which also grew on the chloromycetin plate. Both were recovered from rabbit serum. No strains were recovered on plain blood agar.

Serologic studies with a stock strain of H. ducreyi and the two isolates were attempted. Antisera were prepared in rabbits, using a formalin killed culture. However, despite a variety of techniques, including cultivation in the presence of the surface tension-reducing agent Tween-80, it was not possible to produce a smooth antigen for agglutinin demonstration. To date, other in vitro tests have been unrewarding.

Inactivated seitz filtered human serum from pooled Kahn discards was substituted for rabbit serum in tryptose phosphate broth after the latter had shown successful results. Yields of positive cultures are shown in Table V. Results of cultures with human serum were at least as favorable as those obtained with rabbit serum. The drop in percentage of positive cases in August reflected a wider use of cultural methods in out-patient clinics, with a greater number of questionable lesions being cultured.

Table V. Monthly Trials Of Serum-broth Cultures for H. ducreyi

Month	Total Culture	No. positive for H. ducreyi	% Positive
May	47	34	72.3
June	40	29	72.5
July	45	30	66.7
August	110	69	62.7
September	101	52	51.5
October	105	56	53.3
November	113	60	53.1
December	109	54	49.5

LABORATORY DIAGNOSIS OF TUBERCULOSIS: A comparison of cultures and animal inoculations were made in 3,076 specimens processed during 1951. Of these, 294 (9.6%) were positive for tubercle bacilli. The results are given in Table VI. Of the total positives, 95.2% were obtained by guinea pig inoculation and 70.4% by culture. Corper's medium was positive in 175 (59.4%) of the total positives, although the incubation time was relatively long. Dubos' and Corper's media were almost equally effective. Twenty-nine strains were recovered on Dubos' medium but not on Corper's, while 26 strains which failed to grow on Dubos' medium were recovered on Corper's. Of the 14 cases negative by guinea pig inoculation but positive on culture, 9 were recovered on Dubos' media. Three of these were negative on Corper's. Eleven of the 14 grew on Corper's, 5 of which

were negative on Dubos'. Strains of acid fast bacilli isolated by culture were considered to be non-pathogenic when injection of the culture into a guinea pig produced no infection. There were 53 such isolations, (Table VII) among 3,076 specimens cultured. Of these non-pathogenic acid-fast bacilli, 5 were recovered from both Dubos' and Corper's medium, 44 only from Dubos' and 4 only from Corper's medium. The ease with which non-pathogenic acid-fast organisms propagate in Dubos' liquid medium constitutes a major criticism of this substrate.

Table VI. Comparison of Animal Inoculation and Culture Results
In 294 Specimens Positive For Tuberculosis

Initially Inoculated Guinea Pig	Cultures Dubos'	Corper's	Smear	Number	% of total Positives
+	+	+	+	124	42.1
+	+	+	-	14	4.7
+	-	+	+	6	2.0
+	-	+	-	20	6.8
+	+	-	+	19	6.4
+	+	-	-	10	3.5
+	-	-	+	6	2.0
-	+*	+*	+	5	1.7
-	+*	+*	-	1	0.34
-	+*	-	-	1	0.34
-	+*	-	+	2	0.67
-	-	+*	+	1	0.34
-	-	+*	-	4	1.3

*Cultures confirmed as M. tuberculosis by subsequent animal inoculation

Table VII. Acid-fast Bacilli Recovered in Culture but Non-Pathogenic for Guinea Pigs

Dubos'	Corper's	Smear	No.	% of Total Non-Pathogenic Strains
+	+	+	2	3.7
+	+	-	3	5.6
+	-	+	33	62.2
+	-	-	11	20.7
-	+	-	4	7.5

The results indicated that under the conditions prevailing guinea pig inoculation was the most effective method for recovery of tubercle bacilli. Approximately 95% of the total demonstrated positives were obtained by this method. Dubos' medium and Corper's medium each detected approximately 60% of the total, but together detected about 75% of positives.

Liquid Dubos' medium lacked selectivity, and only 78% of the total acid-fast bacilli recovered in it were tubercle bacilli. Corper's medium although slower to propagate strains, was more reliable, in that 95.2% of acid-fast bacilli recovered were pathogenic.

ACROLEIN PRESUMPTIVE TEST FOR CL. PERFRINGENS: In the 1950 Annual Report (9) the technique for the acrolein reaction (10), (11) was described. During 1951 further experience with this presumptive test for the presence of Cl. perfringens was accumulated. Because of the simplicity of the test and the generally satisfactory results obtained, this procedure was recommended to other laboratories in the theater. The Nagler lecithinase reaction (12) was used for a considerable time, but

on comparison with the acrolein test, results were much more difficult to interpret and the procedure was not only more complicated but slower. Consequently, the Nagler reaction was dropped from routine use.

During 1951 Cl. perfringens was isolated in 134 instances with positive acrolein tests in 118 (88%) and negative in 16 (12%). The acrolein test was positive in 22, equivocal in 4 and negative in 242 of the 268 instances in which Cl. perfringens was not isolated. The 22 false positives represented 15.7% of the total positive tests and 8.8% of the instances in which organisms were not isolated. These results are given in Table VIII.

Table VIII. Results of Acrolein Presumptive Test in 402 Cultures

Acrolein Test	Cl. perfringens Isolated	Cl. perfringens not Isolated
Positive	118	22
Equivocal	0	4
Negative	16	242

Experience with this test showed some of the pitfalls which could cause false reactions. Some of the false negative tests were due to incorrect pH of the test reagent which must be carefully and freshly prepared. In some cases failure to warm the broth to room temperature prior to inoculation appeared to slow the rate of bacterial growth and result in a negative reaction. Although most positive results appeared in 6 hours, some did not occur until after 24 hours. The glycerol broth could not be used for recovery of Clostridia since the acrolein formed was lethal to the organism. It was concluded that this test should be recommended as a presumptive test for the detection of Cl. perfringens.

PROTEUS INHIBITORS IN ANAEROBIC PLATES: To inhibit swarming by Proteus spp. in the routine anaerobic culture procedure, Snyder and Lichstein (13) proposed the use of 0.1% sodium azide. For the same purpose chloral hydrate (0.2%) was proposed by Kramer and Koch (14). In this laboratory chloral hydrate added to blood agar in 0.2% final concentration showed little or no inhibitory effect on either Gram-positive or Gram-negative organisms but tended to inhibit swarming of Proteus. Sodium azide was ineffective for inhibition of swarming below a concentration of 0.2%, although it was useful in inhibiting growth of Pseudomonas at lower levels. Since concentration of either ingredient beyond the stated limit inhibited growth of Clostridial strains a combination of the two reagents was tried. Chloral hydrate and sodium azide, in final concentration of 0.2% each, were used from June to December of 1951. Blood agar plates so treated gave excellent inhibition of Proteus and were superior to plates containing either reagent alone. Hemolysis was suppressed by these inhibitors. For reasons not clear the plates tended to be somewhat dry, so that typical colony morphology was obscured. Therefore, the combined inhibitory agents were used only in conjunction with plain blood agar plates and with 0.2% sodium azide plates, so that colony morphology and hemolysis could be properly evaluated. The plates with combined inhibitors permitted immediate transfer of colonies for fermentation tests and reduced the necessity for purification streaking, thus reducing the time required for identification.

A THIOGLYCOLLATE SEMI-SOLID MEDIUM FOR DEMONSTRATION OF MOTILITY IN CLOSTRIDIA: For several months after the outbreak of the Korean war, the tryptose semi-solid medium suggested by Darby (15) incubated anaerobically was used to determine the motility of Clostridia species. This attribute is important in the identification of Cl. perfringens, the principal non-motile pathogen of this species. To eliminate anaerobic jar incubation, combinations of thioglycollate media similar to those used for carbohydrate fermentation were inoculated with fresh anaerobe isolates and incubated aerobically. The following formula was ultimately adopted.

```
Trypticase (BBL) .......................................   15 gm.
1 - cystine ...........................................   0.75 gm.
Glucose ...............................................   5.0  gm.
Yeast extract .........................................   5.0  gm.
NaCl ..................................................   2.5  gm.
Sodium thioglycollate .................................   0.5  gm.
Agar ..................................................   4.75 gm.
Resazurin .............................................   0.001 gm.
Distilled water .......................................   1000 ml.
```

Loeffler tubes (13 x 70 mm.) containing 6 cc. of the medium were autoclaved at
15 pounds for 15 minutes and stored at room temperature. Tubes were inoculated by
deep stab and were incubated aerobically for 24 hours or longer if necessary at 37°C.
It was unnecessary to reactivate the medium by boiling since the agar concentration
retarded air diffusion for at least 3 weeks. Motility was indicated by a fine haze
growing out from the stab inoculation line. The degree of motility was indicated by
the speed and distance of this growth from the stab line. After this study was com-
pleted it was learned that Mandia (16) has also described a technique for the aerobic
determination of motility of anaerobic organisms with a semisolid broth.

A comparison of the Darby and the thioglycollate media for motility determina-
tions of 102 strains of Clostridia was made. The results are given in Table IX.
Thioglycollate medium has been tested with over 400 members of the genus Clostridium.
Of this series, Cl. tetani, Cl. tertium, Cl. difficile, Cl. sporogenes, Cl. multiferm-
entans, Cl. butyricum and Cl. feseri were actively motile, while Cl. perfringens,
Cl. paraputrificu, and Cl. tetanomorphum were non-motile. Strains of Cl. novyi,
Cl. septique, and Cl. sordelli showed slow, variable motility and several strains
were non-motile.

The two media were approximately of equal value when inoculated with non-motile
and sluggishly motile strains. However, with motile strains the thioglycollate medium
showed motility in 41 of 58 strains (70.7%) while Darby's medium showed motility in
only 23 of these same strains (39.7%). An occasional slight difficulty in reading the
thioglycollate tubes occurred when some strains produced enough gas, apparently due to
glucose fermentation, to split the medium. The enhancement of growth appeared to com-
pensate for the interference however.

The thioglycollate semi-solid medium for testing motility of Clostridia proved to
be more satisfactory than methods employed earlier. In addition it was found that when
overlaid with mineral oil, cultures remained viable for at least 8 months and the
medium thus provided a method for maintaining stock cultures.

Table IX. Motility of Clostridia as Shown by Darby's and
Thioglycollate Motility Media

Organism	Darby's Medium				Thioglycollate Motility Medium			
	Non-Motile	Motile	Doubt-ful	No Growth	Non-Motile	Motile	Doubt-ful	No growth
Non-Motile								
Cl. perfringens	19	0	0	0	19	0	0	0
Cl. paraputrificum	2	0	0	0	2	0	0	0
Cl. tetanomorphum	1	1	0	0	1	0	0	0
Sluggishly Motile								
Cl. novyi	3	0	1	0	3	0	1	0
Cl. septique	1	0	0	0	1	0	0	0
Cl. sordelli	0	1	0	0	1	0	0	0
Motile								
Cl. tetani	0	3	0	0	0	3	0	0
Cl. tertium	1	0	0	0	1	0	0	0
Cl. difficile	1	0	0	0	0	1	0	0
Cl. sporogenes	13	19	1	5	4	30	1	3
Cl. multifermentans	4	0	0	0	2	2	0	0
Cl. bifermentans	4	0	0	2	4	0	0	2
Cl. butyricum	1	1	0	0	0	2	0	0
Cl. feseri	2	0	0	1	0	3	0	0
Unclassified	7	6	1	1	3	12	0	0
Total	59	31	3	9	42	53	2	5

SEMI-SOLID THIOGLYCOLLATE NITRATE BROTH FOR DEMONSTRATION OF NITRATE REDUCTION BY ANAEROBES: Standard nitrate broth and agar media methods (17) for demonstrating nitrate reduction by anaerobes have the following disadvantages: they require anaerobic incubation, occasionally give false positive results after 5 to 14 days storage even when not inoculated, and though the solid agar gives fewer equivocal and false positive results, some strains grow poorly on slants. A thioglycollate nitrate semi-solid broth was devised in an attempt to overcome these handicaps.

Some reduction of nitrate to nitrite occurred when an excess of sodium thioglycollate was added to nitrate in aqueous solution but this appeared to be absent in the semi-solid medium. Repeated tests of thioglycollate-nitrate broth after periods of storage up to three weeks at room temperature showed no color change on addition of reagents. The maintenance of adequately low-oxidation-reduction potential in this medium was indicated by the resazurin. This indicator did not, however, obscure the results of the test. The formula finally used is given below. All ingredients except the reazurin were dissolved in water, the indicator was then added, the medium was tubed in 5 cc. amounts in sterile Wassermann tubes and the batch was immediately autoclaved at 10 pounds for 10 minutes.

Thioglycollate nitrate medium:

KNO_3 .... 1 gm.
Tryptose .... 10 gm.
NaCl .... 5 gm.
Reazurin .... 0.001 gm.
Water .... 1000 ml.

Thioglycollate nitrate medium was employed in a parallel study with nitrate broth and nitrate agar, the two latter media being incubated anaerobically in Brewer jars. Batches of nitrate broth employed were checked to be sure than uninoculated tubes did not give a positive reaction. Six species of recently isolated Clostridia, comprising a total of 35 strains, were studied with results as shown in Table X.

As seen in Table X the thioglycollate-nitrate semi-solid medium was at least as satisfactory as other standard media, gave fewer false negative and equivocal reactions and did not inhibit growth of the organisms. This medium has been used in over 200 routine cultures for Clostridia in wounds and has functioned efficiently without apparent discrepancies.

ANAEROBIC BACTERIAL FLORA OF WAR WOUNDS IN THE FAR EAST COMMAND IN 1951: The distribution of Clostridia in wounds in the Korean War has been observed in 420 cultures received by this laboratory during 1951 for isolation and/or identification of anaerobes. The clinical history and description of the injury were unavailable. However, the bacteriologic data was of significance in evaluation of laboratory procedures for isolation of pathogenic anaerobes, and a reasonably correct representation of the incidence of Clostridia in wounds in the Korean War was obtained.

Clostridia were isolated from 299 (71%) of the 420 specimens submitted. Specimens were received in the form of thioglycollate broth cultures, cooked meat broth cultures, tissue fragments, swabs and body fluids. The recovery rate for all of these methods except the swab method of transmitting material for isolation of Clostridia proved satisfactory. The relative recovery rate is summarized in Table XI. The incidence of the 23 different strains of Clostridia is given in Table XII.

The total of 480 strains isolated from 299 positive specimens submitted was equivalent to 1.6 strains per specimen. This is less than the figure of 2.84 per case described by Smith (18), of 2.56 per case found by Stock (19), or of 2.62 per case by MacLennan (20). However, these investigators were closer to the clinical material, and did not rely on long-distance transport of cultures in enrichment media. In 40 cases in our series material was collected by a bacteriologist in close collaboration with the pathologist and the number of strains recovered rose to 2.15 per case. Of Clostridial species found, Cl. feseri has not previously been reported as a human tissue pathogen, even in the studies of wound anaerobes during World War II as quoted above. The

Table X. Comparison of Thioglycollate-nitrate Medium With Nitrate
Broth and Nitrate Agar

Species	No. of Strains	Reaction		
		Thioglycollate-Nitrate Medium	Nitrate Broth	Nitrate Agar
Cl. perfringens	8	4∕	4∕	4∕
	1	4∕	2∕	4∕
	1	4∕	1∕	4∕
	1	4∕	4∕	2∕
Cl. tetani	1	-	-	-
	2	-	1∕	-
	1	-	∤	-
	1	-	2∕	-
Cl. bifermentans	7	-	-	-
	1	-	∤	-
Cl. novyi	1	-	-	-
	1	-	1∕	-
Cl. sporogenes	3	-	-	-
	1	-	1∕	-
	1	-	∤	-
Cl. putrificum	2	-	-	-
	2	-	1∕	-
Controls:				
Negative spp.	6	-	∤	-
Positive spp.	6	4∕	4∕	4∕

Table XI. Recovery of Clostridia from Varying Types of Specimens

Specimen Submitted	No. of Cases	Clostridia Isolated	% Positive Isolations
Thioglycollate broth	147	112	76.2
Cooked meat broth	60	42	70.0
Tissue	191	136	71.8
Swabs	10	1	10.0
Body fluids	7	3	42.9
Culture for identification on Semi-solid agar	5	5	
	420	299	

relatively low incidence of Cl. novyi is unusual since this species was much more common in Europe and North Africa. Unidentified strains of Clostridia were also less frequently encountered (5.2%) than in World War II (15%).

A limited study of the aerobic flora of wound specimens submitted for anaerobic culture was made. Cultures from 150 tissues yielded 518 strains of aerobic bacteria after plating from thioglycollate or cooked meat broth. An average of 3.45 strains per specimen was present. Table XIII shows the species distribution of these strains.

The predominant species in this series were Staphylococci and Streptococci, which together contributed 44% of the total flora. Smith, in 1949, noted a 35% incidence of

Table XII. Species of Clostridium Isolated

Species	No.	% of Total Recovered
Cl. sporogenes	149	31.0
Cl. perfringens	139	28.9
Cl. lentoputrescens	42	8.7
Cl. novyi	27	5.6
Cl. bifermentans	22	4.6
Cl. multifermentans	20	4.1
Cl. tetani	13	2.7
Cl. tetanonorphum	11	2.3
Cl. cochlearium	6	1.3
Cl. tertium	5	1.0
Cl. sordelli	4	0.8
Cl. fesseri (chauvaei)	3	0.6
Cl. paraputrificum	2	0.4
Cl. fallax	2	0.4
Cl. innominatum	2	0.4
Cl. butyricum	2	0.4
Cl. capitovalis	1	0.2
Cl. histolyticum	1	0.2
Cl. carnis	1	0.2
Cl. septicum	1	0.2
Cl. aerofaetidum	1	0.2
Unclassified Clostridia	25	5.2
Total	480	

Table XIII. Species of Aerobic Organisms Recovered From Tissues Amputated For Battle Injury

Species	No.	% of Total
Staphylococcus, non-hemolytic	49	9.4
Staphylococcus, hemolytic	69	13.1
Streptococcus, alpha-hemolytic	18	3.4
Streptococcus, beta-hemolytic	37	7.1
Streptococcus, non-hemolytic	55	10.6
Enterococcus, alpha-hemolytic	1	0.2
Enterococcus, beta-hemolytic	9	1.7
Enterococcus, non-hemolytic	6	1.1
Neisseria flava	1	0.2
Escherichia coli	24	4.6
Escherichia freundii	9	1.7
Aerobacter aerogenes	34	6.6
Alcaligenes spp.	38	7.3
Paracolobactrum aerogenoides	26	5.0
Paracolobactrum coliforme	2	0.4
Paracolobactrum spp.	18	3.4
B. anitratum	3	0.6
Proteus spp.	39	7.5
Pseudomonas spp.	25	4.8
B. subtilis	26	5.0
Corynebacterium spp.	19	3.6
Sarcinae spp.	3	0.6
Serratia marcescens	7	1.3

these genera in a series of wound cases, but a much lower incidence of enteric bacilli than were found here. From the outset of the Korean War it has been assumed that the

enteric bacterial flora would play a prominent role in wounds, because of the large amount of fecal pollution in the cultivated soil of the country. Organisms of fecal origin comprised 32% of the aerobic flora of these tissues. Proteus and Pseudomonas strains, which are commonly encountered in contaminated wounds, were relatively infrequent in this series.

DISTRIBUTION OF ANAEROBIC AND AEROBIC FLORA AND OF ANTIBIOTIC SUBSTANCE IN TISSUES OF TRAUMATIC GANGRENE CASES: Distribution of bacteria in infected tissues and the relationships of antibiotic content of such tissues to bacterial flora can seldom be studied extensively in clinical material, since such tissues do not often become available. Determinations of penicillin levels in the body have been principally devoted to study of body fluid levels. Cutting (21) demonstrated striated muscle content of penicillin approximating that in blood in experimental animals. Struble and Bellows (22) reported smaller proportions of antibiotic in muscle than in serum. It has been suggested that reducing substances in disintegrating tissue may inactivate penicillin. Chemotherapeutic agents have not prevented initiation of Clostridial infection (23), (24).

A study of the presence of both aerobes and anaerobes, and of tissue content of antibiotic, was made on tissue from extremities amputated for gangrene chiefly secondary to battle injury and frostbote. Wounded soldiers in Korea and at all medical installations in the chain of evacuation routinely received extensive penicillin therapy. The level of antibiotic in tissues therefore was determined in terms of penicillin only. Other inhibitors, potentially present, were not identified. Only tissues were studied since circumstances did not permit determination of blood levels of antibiotic in patients prior to operation.

Tissues were planted in cooked meat broth for 24 hours, at which time aerobic and anaerobic sub-cultures were made (9). Sodium azide-blood agar plates and thioglycollate-glycerol broth were inoculated from the initial broth (13). The azide-blood agar plates were incubated anaerobically 48 hours in a Brewer jar, then picked for potential anaerobe colonies. Confirmation of anaerobic requirements of isolates was made on an aerobic blood agar plate, after which complete identification was carried out by biochemical reactions and animal inoculation.

ASSAY OF ANTIBIOTIC IN MUSCLE TISSUES: Experiments to devise an accurate and convenient method for assay of penicillin in muscle tissue were made using guinea pigs and rabbits injected with suitably proportioned doses of this antibiotic. An agar-well technique and a modified absorbent-disc technique (25) for applying tissue extract to seeded plates gave minimum readable limits of 0.4 units per gram and 0.25 units per gram of tissue respectively. Emulsified human gangrenous tissue frequently required greater amounts of extracting solution than normal animal tissue. Such dilution made it necessary to use a more sensitive assay method then those described. Stainless steel cylinders 10 mm. high, with an inside diameter of 10 mm., were used to permit 0.5 ml. or more of tissue extract to be applied to a seeded blood agar plate containing 15 ml. of 2.0% agar. Tissue blocks were weighed, ground with sand in a sterile mortar and isotonic saline was added to the tissue paste. Most gangrenous tissues required 3 volumes of saline in order to obtain a usable amount of extract. Tissue suspensions were extracted in the cold for 20 minutes and centrifuged. The extract was, in most cases, passed through a Seitz microfilter to avoid subsequent effect of growth of bacteria present in the extract. The test organism was the C-203-MV strain of hemolytic streptococcus, which is one of the most sensitive of standard penicillin assay strains. This organism was found not to be inhibited by normal serum or normal tissue extracts under conditions of the test. Four cylinders were placed on each plate after the surface had been seeded with an 18 hour broth culture of streptococcus and dried for 20 minutes. In each of two cylinders 0.5 ml. of tissue extract was placed and in the remaining two were placed 0.5 ml. of two control solutions of penicillin in 10% normal serum, the concentrations being 0.025 units/ml. and 0.20 units/ml. Plates were incubated at 37°C. for 24 hours and read. A 4-dilution control plate, as indicated below, was included with each test. Dilution factors varied with different tissues, and were calculated individually.

A standard inhibition zone curve and range of error at significant penicillin levels were calculated from a graded series of observations. Derivation of the curve is shown in Table XIV.

Table XIV.   Derivation of Standard Tissue-Penicillin Curve

Penicillin Content (u/ml.)	No. of Observations	Range of Diameter of inhibition zone in mm.	Mean of Diameter of Inhibition zone in mm.	Standard Deviation
0.025	12	less then 11	-	-
0.05	12	11.0 - 13.5	12.0	0.58
0.10	11	13.0 - 15.0	14.0	0.52
0.20	10	15.0 - 17.0	16.0	0.50
0.40	12	16.5 - 18.5	17.5	0.54
0.80	11	18.0 - 20.5	19.5	0.63

Figure 1 shows the graphic representation of data from which penicillin content of tissues may be derived. Each test included four standard dilutions of 0.025 u/ml., 0.05 u/ml., 0.1 ml., and 0.2 u/ml., on a control plate. Tissue levels of inhibitor represented at least duplicate determinations. Throughout the study the inhibiting level was determined in terms of penicillin. However, there was no absolute basis for stating that the substance demonstrated was in fact penicillin.

Individual human amputation specimens were dissected promptly as received by staff members of the Pathology Department. Surfaces were sponged with iodine and 70% alcohol. An incision was made with a sterile scalpel and, with a fresh scalpel and forceps, a block of tissue approximately 1.5 cm x 0.5 cm x 2 cm was removed aseptically.

The tissue was divided into portions for culture, penicillin assay, and for histologic examination. Distribution of samples, which ranged in number from 2 to 8 (average 2.8) per amputated extremity, was such as to include relatively healthy tissue at the amputation level and from one to several blocks below the level of amputation, extending into gangrenous areas.

The volume of anaerobic culture work necessitated storage of many specimens at -20°C. to -50°C. in deep-freeze. Parallel determinations showed no loss in antibiotic activity due to this practice and, except for a possible reduction in the percentage of recoveries of Cl. perfringens, the bacterial content of tissues was not significantly altered (26). When experimental animal tissues were assayed for penicillin content, the levels were not appreciably reduced by storage in the frozen state.

A total of 28 cases were cultured and assayed for antibiotic content of tissues. Of these, 20 were designated as gangrene secondary to missile wounds, six were frostbite injuries, and two were primarily vascular damage due to accident. Table XV shows distribution of anaerobic flora and of antibiotic levels in the tissues from the missile wound cases and Table XVI gives the same data for the frostbite and vascular damage cases. The total range of aerobic flora per case is included, but not itemized for individual tissue blocks.

Eleven of the 20 missile wound cases (55%) showed pathogenic Clostridia, including Cl. perfringens, Cl. novyi, Cl. sordelli, Cl. fallax, and Cl. tetani. Ten species of non-pathogenic Clostridia (listed in Table XVII also occurred in this group. Four cases yielded no anaerobic organisms. From the table it is apparent that antibiotic activity was not symmetrically distributed in a centripetal manner. The average antibiotic levels from proximal tissue blocks was 0.26 u/gm. of tissue compared with an average level from the most distal samples of 0.22 u/gm. of tissue. This difference cannot be considered significant. The point at which the greatest concentration of antibiotic was present was then considered. In six instances, levels were the same throughout. In the remaining 14, the highest antibiotic level was found in 6 cases in the proximal block, in 3 cases in a median block, and in 5 cases in a distal block. The average level of antibiotic in all tissues of this group was 0.23 u/gm. All of the 6 frostbite cases yielded Clostridia,
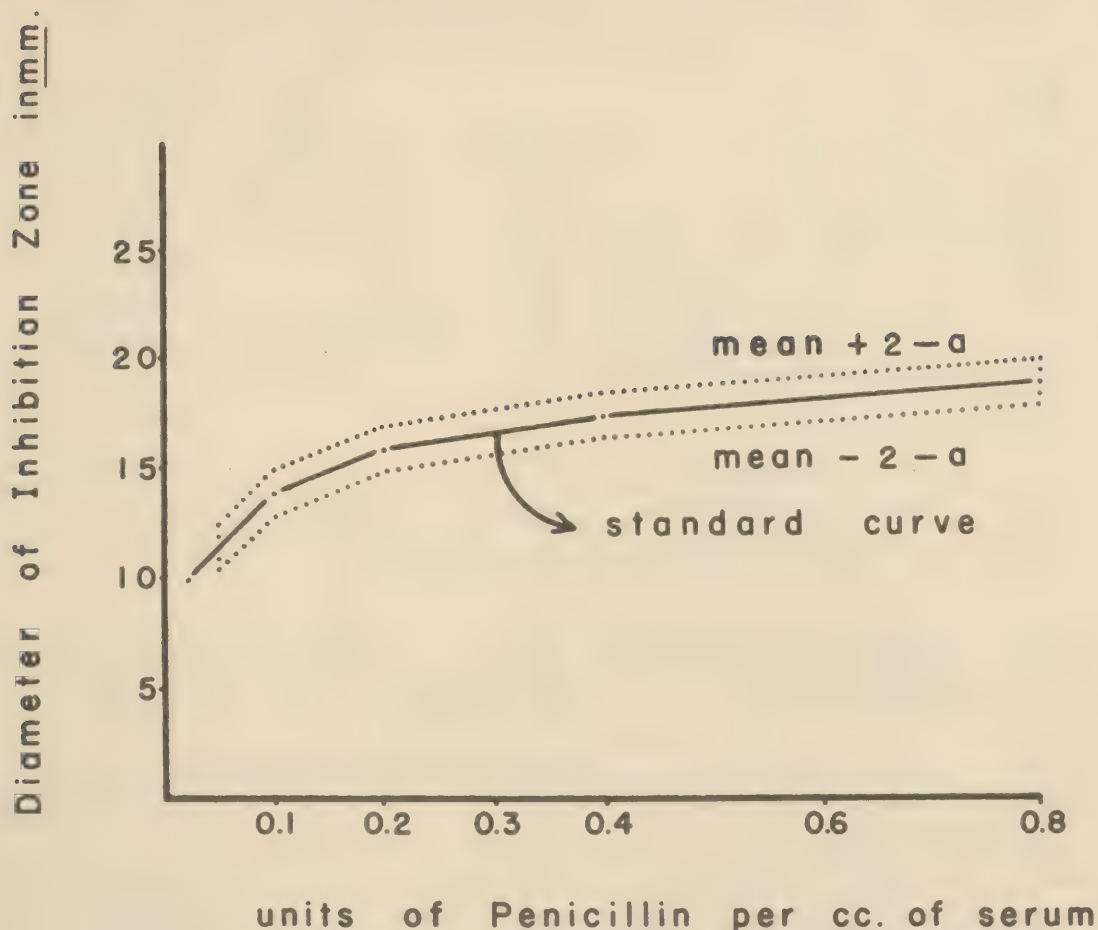
Figure 1. Expected Variation In Penicillin Content Determined From Zone Diameter

but in only 2 cases were pathogenic species recovered. No anaerobes were recovered from the strictly vascular-damage cases. The mean level of antibiotic in frostbite case tissues was lower than in the traumatic cases, and ranged from 0.05 u/gm. to 0.25 u/gm.

The distribution of Clostridia in tissue blocks and the relation of this flora to an empirically selected level of 0.2 u/gm. of antibiotic (expressed as penicillin) in tissues, is shown in Table XVII. Traumatic and cold-injury tissues are included. The distribution of pathogenic Clostridia was virtually the same in both groups of tissues. The total recoveries do not justify detailed conclusions regarding effect of antibiotic on tissue concentrations of anaerobes.

An additional nine cases of gangrene in wound cases were cultured for deter- mination of bacteria in tissues, although no tissue antibiotic levels were obtained. Table XVIII summarizes the distribution of organisms in these tissues. As was seen in the 20 cases presented in Table XV, no consistent correlation between anatomic region and anaerobic or aerobic flora was apparent. Pathogenic strains and non- pathogenic strains were found in tissue at the site of amputation, midway between amputation, at the distal end of extremities, and in frankly decomposed tissue.

Multiple infection is usual in clostridial infections. The current series showed in 29 cases of injuries due to gunshot wounds, 4 instances in which no Clostridia were recovered, 6 yielded 1 species, 5 with 2 species, 6 with three species, 3 with four species, 4 with five species and 1 case with eight species of Clostridia. Of

Table XV. Distribution of Anaerobes, Antibiotic Levels and Aerobe Flora In Tissues Of Battle Wound Cases

Case No.	Tissue Block No.	Tissue (source)	Anaerobes	Antib. Level u/gm.	Aerobes Present (total for all tissues)
1	1108	Leg: popliteal muscle, medial	None	0.56	Staph., H⁄, H- *
	1109	Leg: calf muscle above incision	None	0.64	alpha, beta, and gamma strep.
	1110	Leg: muscle lateral to wound	Cl. perfringens	0.4	C. hoffmanni and C. xerosis
	1111	Leg: antero-medial aspect above malleolus	None	0.4	A. fecalis
	1112	Foot: distal area, discolored	None	0.1	B. subtilis
	1113	Leg: medial aspect, 2 cm above incision	None	0.4	S. marcessens
	1114	Leg: cut muscle at site of amputation	None	3.2	
	1115	Leg: liquified soleus muscle	Cl. perfringens		
2	1514	Leg: necrotic muscle	None	0.3	Staph., H⁄
	1515	Leg: ankle	None	0.2	beta Strep.
	1516	Foot: dorsum	Cl. sporogenes Cl. putrificum	0.2	A. aerogenes
3	1931	Leg: gangrenous area	None	0.32	gamma Strep.
	1932	Leg: amputation site	None	0.08	Proteus mirabilis
4	2144	Leg: amputation site	None	0.10	Staph., H⁄, H-
	2145	Leg: proteolysed muscle, below incision	None	0.05	beta and gamma Strep. C. hoffmanni and
	2146	Foot: dorsum	None	0.05	C. xerosis
	2147	Leg: above ankle	None	0.05	Alcaligenes sp. Ps. aeruginosa B. subtilis
5	2379	Leg: re-amputation; border of amputation	Cl. perfringens Cl. sporogenes Cl. unidentified	0.08 0.12	Staph., H⁄ alpha and gamma Strep Paracolon spp.
	2380	Leg: edge of wound	Cl. perfringens Cl. putrificum	0.12	Ps. aeruginosa Alcaligenes spp.
	2381	Leg: liquified tissue	Cl. sporogenes	0.05	E. coli B. subtilis
6	2588	Leg: below amputation	Cl. perfringens	0.05	A. aerogenes
	2589	Leg: non viable area	Cl. perfringens	0.05	Pr. mirabilis
	2590	Leg: dorsum, foot	Cl. tetanomorphum	0.10	B. subtilis
7	2700	Leg: upper leg	None	0.0	Non-hemolytic
	2701	Leg: midcalf	None	0.0	Staph., H⁄, H-
	2702	Foot: medial aspect	None	0.05	alpha, beta and gamma Strep. Alcaligenes spp. B. subtilis

Table XV. Distribution of Anaerobes, Antibiotic Levels and Aerobe Flora
In Tissues of Battle Wound Cases Continued

Case No.	Tissue Block No.	Tissue (source)	Anaerobes	Antib. Level u/gm.	Aerobes present (total for all tissues)
8	2762	Leg: viable muscle	None	0.08	C. haffmanni
	2763	Leg: viable muscle below wound	Cl. novyi	0.02	Alcaligenes spp.
			Cl. tertium		Esch. freundii
			Cl. innominatum		
9	3130	Leg: gangrene area	Cl. sporogenes	0.2	Staph., H≠, H-
	3131	Leg: site of amputation	Cl. perfringens	0.2	gamma Strep.
					Proteus mirabilis
					A. aerogenes
					Paracolon spp.
10	3435	Leg: site of amputation	None	0.3	B. subtilis
	3436	Leg: muscle of calf	Cl. bifermentans	0.3	Alc. fecalis
			Cl. sporogenes		
			Cl. unidentified		
11	4312	Arm: thru skin	Cl. sporogenes	0.40	Staph., H≠
	4313	Arm: necrotic flexor	Cl. sporogenes	0.05	beta Strep.
	4314	Arm: hand	None	0.40	Paracolon spp.
					A. aerogenes
12	4404	Leg: amputation site	None	0.20	Staph, H≠
	4405	Leg: calf muscles	Cl. sporogenes	0.20	beta Strep.
					A. aerogenes
					Ps. aeruginosa
13	4416	Leg: edge of wound	None	0.10	Staph., H≠
	4417	Leg: site of amputation	Cl. sporogenes	0.18	beta and gamma Strep.
	4418	Leg: foot	Cl. sporogenes	0.09	A. aerogenes
					E. freundii
14	4808	Leg: muscle above	None	0.35	Staph., H≠, H-
	4809	Leg: site of amputation	None	0.40	gamma Strep.
	4810	Leg: foot	None	0.60	Alcaligenes spp.
					Paracolon spp.
15	4962	Leg: site of amputa-	Cl. multiferment.	0.05	Staph., H≠, H-
			Cl. sporogenes		beta and gamma Strep.
			Cl. perfringens		A. aerogenes
	4963	Leg: below wound	Cl. sporogenes	0.05	
			Cl. multifermen-tans		
			Cl. tetanomorphum		
	4964	Leg: foot	Cl. multiferment	0.0	
			Cl. sporogenes		
			Cl. butyricum		
16	5228	Leg: foot, viable muscle	Cl. multiferment.	0.10	Staph., H-
			Cl. tetanomorphum		gamma Strep.
			Cl. sporogenes		A. aerogenes
			Cl. perfringens		Paracolon spp.
	5229	Leg: calf muscle	Cl. paraputrifi-cum	0.10	E. coli

Case No.	Tissue Block No.	Tissue (source)	Anaerobes	Antib. Level u/gm.	Aerobes present (total for all tissues)
17	8021	Leg: site of amputation	None	0.05	Staph., H≠, H-gamma Strep.
	8022	Leg: below incision	Cl. sporogenes	0.4	N. flava
	8023	Leg: necrotic tissue	Cl. perfringens Cl. tetani	0.45	A. fecalis C. serosis
	8024	Leg: necrotic tissue calf	Cl. novyi Cl. sporogenes	0.2 0.2	B. subtilis Paracolon spp.
	8025	Leg: foot	Cl. tetani Cl. sporogenes	0.1	A. aerogenes Pseudomonas spp.
18	8601	Leg: foot, viable	Cl. novyi Cl. multiferment. Cl. bifermentans Cl. sporogenes	0.9	Staph., H-alpha and gamma Strep. Strep. fecalis C. hoffmanni
	8602	Leg: sub-cut tissue ankle, posterior	Cl. multiferment. Cl. sordelli Cl. sporogenes	0.1	Paracolon spp. A. aerogenes E. coli
	8603	Leg: granulation tissue medial to wound	Cl. novyi Cl. tetani Cl. fallax	0.2	B. subtilis
	8604	Leg: anterior wound, grossly contaminated	Cl. perfringens Cl. putrificum	0.1	
	8605	Leg: muscle, site of amputation	Cl. sporogenes		
	8606	Leg: between gastrocnemius and soleus muscles	Cl. putrificum	0.5	
19	8608	Leg: amput. site	Cl. novyi	⟩0.05	Staph., H-alpha and gamma Strep.
	8609	Leg: granulation tissue; gas present	Cl. perfringens	0.1	Strep. fecalis
	8611	Leg: lower calf	None	0.1	C. xerosis
	8610	Leg: lower calf	Cl. bifermentans Cl. sporogenes Cl. novyi	⟩0.05	A. aerogenes B. anitratum Pseudomonas spp.
	8612	Leg: at wound	Cl. sporogenes Cl. bifermentans Cl. sordelli Cl. novyi	0.05	Staph., H≠, H-beta and gamma Strep. A. aerogenes E. coli
	8613	Leg: above ankle	None	0.18	Paracolon spp.
	8614	Leg: calf muscle: site of amputation	None	0.10	Alcaligenes spp.
	8615	Leg: below wound	Cl. novyi	0.05	

* H≠ - hemolytic
H- - non-hemolytic

Controls   (Penicillin-free Tissue Blocks)

A	10 blocks	Normal muscle	0
B	5 blocks	Gangrenous human muscle (vascular damage)	0
C	15 blocks	Gangrene: animal muscle	0

Table XVI. Distribution of Anaerobes, Antibiotic Levels, and Aerobes
In Tissues of Frostbite and Vascular Damage Cases

Case No.	Tissue Block No. Frostbite	Tissue (source)	Anaerobes	Antib. Level u/gm.	Aerobes
21	2480	Leg: calf	Cl. sporogenes	0.05	Staph., H≠
	2481	Leg: above ischemic zone	Cl. putrificum	0.1	Proteus mirabilis
22	2691	Leg: site of amputation	Cl. sporogenes	0.05	Staph., H≠, H-
			Cl. tetanomorphum		Pr. morgani
	2692	Leg: margin of wound	Cl. tetani	0.05	A. aerogenes
			Cl. sporogenes		
	2693	Leg: calf, muscle	Cl. novyi	0.05	
	2694	Leg: dorsum of foot	Cl. sporogenes	0.05	
23	3089	Leg: calf	None	0.25	Staph., H≠, H-
	3090	Leg: foot	Cl. sporogenes		gamma Strep.
					C. xerosis
					Alcaligenes spp.
24	3081	Leg: site of amputation	Cl. perfringens	0.2	No aerobic culture
	3082	Leg: below viable area	Cl. perfringens	0.1	
			Cl. multiferment.		
25	3267	Leg: site of amputation	Cl. bifermentans	0.2	Staph., H≠
	3268	Leg: lower calf	Cl. sporogenes	0.05	alpha, beta, and
					gamma Strep.
					Alcaligenes spp.
					Proteus mirabilis
26	8622	Leg: site of amputation	None	0.09	Staph., H≠, H-
	8623	Leg: calf, muscle	None	0.20	alpha and gamma
	8624	Leg: foot	Cl. putrificum	0.05	Strep.
					Alcaligenes sp.
					A. aerogenes
					Paracolon spp.
					Pseudomonas aerug-inosa
					E. coli
27	1158	Muscle over elbow	None	0.4	Staph., H-
	1159	Muscle edge of wound	None	0.16	Paracolon spp.
	1160	Dorsum of lower arm	None	0.05	Alc. fecalis
					B. subtilis
28	3091	Arm: at site of amput-	None	0.1	Staph., H≠
	3092	Arm: hand ation	None	0.1	beta Strep.
					Alcaligenes spp.
					C. hoffmanni
					A. aerogenes
					Paracolon spp.

Table XVII. Relation Between Presence Of Clostridia and Presence
of Antibiotic in Tissue

Species	0.2 or more antibiotic u/gm. of tissue	0 to 0.19 antibiotic u/gm. of tissue	Total
Pathogens:			
Cl. perfringens	4	10	14
Cl. novyi	3	6	9
Cl. sordelli	0	2	2
Cl. fallax	1	0	1
Cl. tetani	2	2	4
Non-Pathogens			
Cl. sporogenes	10	18	28
Cl. putrificum	1	5	6
Cl. paraputrificum	0	1	1
Cl. tetanomorphum	0	4	4
Cl. tertium	0	1	1
Cl. inominatum	0	1	1
Cl. multifermentans	1	6	7
Cl. bifermentans	3	2	5
Cl. butyricum	0	1	1
Unidentified Clostridia	1	1	2
Clostridia not present	16	16	32
Total	42	76	118

Table XVIII. Regional Distribution of Bacteria in 9 Cases of Gangrene
Due to Gunshot Wound

Case No.	Tissue Block No.	Tissue (source)	Anaerobes	Aerobes
29	518	Leg: midcalf	Cl. perfringens	Staph., H∕, H- *
	519	Leg: foot	None	alpha and gamma Strep.
				C. xerosis
				Paracolon spp.
				B. subtilis
30	3080	Leg: site of amputation	Cl. perfringens	Staph., H∕
	3081	Leg: site of amputation	Cl. novyi	alpha and gamma Strep.
			Cl. tetanomorphum	C. hoffmanni
	3082	Leg: site of wound, calf	Cl. perfringens	A. aerogenes
	3083	Leg: 5 cm. above malleolus	Cl. perfringens	E. coli
	3084	Leg: subcut. tissue above ankle	Cl. perfringens	B. anitratum
				B. subtilis
	3085	Leg: dorsum of foot	Cl. novyi	
	3086	Leg: calf	Cl. perfringens	
			Cl. multifermentans	
	3087	Leg: calf, muscle	Cl. sporogenes	
31	6865	Leg: wound below amputation	Cl. putrificum	Staph., H∕
	6866	Leg: amputation site	Cl. putrificum	A. aerogenes
	6867	Leg: below wound	Cl. putrificum	B. subtilis
	6868	Leg: lower calf	Cl. perfringens	
	6869	Leg: above ankle	Cl. putrificum	
			Cl. unidentified	

Case No.	Tissue Block No.	Tissue (source)	Anaerobes	Aerobes
32	6881	Leg: tissue at wound	Cl. perfringens Cl. novyi	Staph., H∕ alpha Strep.
	6882	Leg: site of amputation	Cl. perfringens	A. aerogenes
	6883	Leg: deep calf, muscle	Cl. perfringens Cl. sporogenes	E. coli
	6885	Leg: foot	Cl. sporogenes	
33	6894	Leg: site of amputation	Cl. sporogenes Cl. putrificum	alpha Strep. Proteus sp.
	6895	Leg: below wound	Cl. perfringens	Paracolon spp.
	6896	Leg: necrotic tissue, lower calf	Cl. novyi Cl. putrificum Cl. perfringens	E. coli
	6897	Leg: foot	Cl. novyi	
	6898	Leg: deep muscle, calf	Cl. histolyticum	
34	6970	Leg: site of amputation	None	alpha Strep.
	6971	Leg: calf muscle	None	Proteus spp.
	6972	Leg: edge of wound	None	Paracolon spp.
	6973	Leg: deep muscle	Cl. putrificum	E. coli
	6974	Leg: ankle	None	
	6975	Leg: foot	Cl. putrificum	
	6976	Leg: gastrocnemius	Cl. putrificum	
35	7406	Leg: site of amputation	Cl. unidentified	Staph., H∕
	7407	Leg: above ankle	Cl. feseri Cl. sporogenes Cl. unidentified	Pr. morgani B. subtilis A. fecalis
36	10013	Leg: site of amputation	None	Staph., H∕
	10014	Leg: above ankle	Cl. sporogenes Cl. novyi	beta and gamma Strep. Proteus sp. E. coli
37	16501	Foot at amputation site	Cl. novyi Cl. sporogenes	No aerobic culture
	16502	Foot below amputation site	Cl. sporogenes	

* H∕ - hemolytic
H- - non-hemolytic

104 tissue blocks, 35 contained no Clostridia, 39 portions yielded 1 speciea, 16 yielded 2 species, 11 contained 3 species, and 3 had 4 different species present. Six cases of gangrene due primarily to frostbite included 2 cases with 1 species present, 3 with 2 species, and 1 with 4 species. Individual tissue samples in the frostbite series included 3 with no Clostridia, 7 with 1 species, and 3 with 2 species of Clostridia.

AEROBIC FLORA: The significance of the association of aerobic and anaerobic flora in wounds is not well understood. The literature on this subject covers principally observations prior to the advent of antibiotics. The species shown in Tables XV, XVI, XVIII illustrate the complexity of the bacterial population.

Aerobic organisms recovered from 85 tissue blocks obtained from 26 patients are listed in Table XIX together with observations on penicillin sensitivity of these organisms. No significant correlation between penicillin sensitivity and aerobic and anaerobic flora was noted. The numbers of aerobes increased at greater distances from the site of amputation. Since penicillin therapy was intensively employed in these patients, the presence of large numbers of Streptococci and Staphylococci was somewhat

unexpected. Sensitivity of the strains of aerobes to one unit of penicillin per cc. of media was determined using a method in which the antibiotic was incorporated in agar. Sensitive strains included 2/3 of the hemolytic streptococci, 2/3 of the non-hemolytic staphylococci, and a majority of the Corynebacteria. Hemolytic staphylococci were closely divided between sensitive and resistant forms, while 3/4 of the B. subtilis strains were resistant. With two exceptions all gram-negative bacilli were resistant. Penicillin-sensitive organisms were recovered from both viable and non-viable muscle tissue.

It has been reported (21), (22), that voluntary muscle is low in amount of antibiotic absorbed during therapeutic administration.

Table XIX. Aerobic Bacteria Recovered From 85 Blocks of Tissue From 26 Patients With Penicillin Sensitivity of 206 Of the 283 Strains Recovered

Organism	No. of Patients with Positive cultures	No. of Tissue Blocks with Positive Cultures	Strains sensitive to 1 unit Penicillin/ml	Strains resistant to 1 unit Penicillin/ml
A. aerogenes	12	23	0	12
Alcaligenes spp.	11	21	1	9
E. coli and freundii	8	14	0	14
Paracolon sp.	4	4	0	4
Para. aerogenoides	10	13	0	8
Para coliforme	1	1	0	1
Proteus spp.	7	11	0	5
Pseudomonas spp.	7	12	1	1
Serratia spp.	8	13	4	2
B. subtilis	11	20	3	11
Corynebacterium spp.	7	12	5	1
Sarcina spp.	1	4	2	2
Strep., alpha-hemolytic	6	15	6	9
Strep., beta-hemolytic	10	13	6	3
Strep., non-hemolytic	18	33	15	18
Enterococci*	7	13	3	6
Staphylococci, hemo.	16	31	15	18
Staph. non-hemolytic	14	22	10	6
B. anitratum	4	4	0	4
Neisseria spp.	2	2	0	1
			71	135

* Differentiated from Streptococci biochemically

Penicillin substantially disappears within one hour after a single massive intravenous injection. Persistence may be enhanced by depot penicillin administration but is in any event not greatly prolonged.

It was known that from 300,000 units to 1,000,000 units of penicillin per day was routinely employed in virtually all Korean battle casualties. It was assumed that tissues of such patients might show residual penicillin from such therapy. The presence of antibiotic in viable, non-viable, and even necrotic muscle was confirmed. Although the concentrations are highest at the site of amputation, and tended to be lower distally, they were low in all cases. Only one tissue block showed a level of over 1.0 unit/gm. Whether the antibiotic action was due to penicillin or to a combination of penicillin and some other agent was not determined. Control studies indicated that extracts of both normal and necrotic tissue did not inhibit growth of the test organism, while tissues from penicillin-treated animals consistently yielded inhibitory extracts indicating that such action was due to the concentration of penicillin in the muscle.

INCIDENCE AND DISTRIBUTION OF SHIGELLA TYPES IN JAPAN AND KOREA IN 1951: The distribution of Shigella species and types is of continuous epidemiologic interest. Such data has become increasingly significant since specific serologic means of identification have become available. This was based on the work of numerous investigators (27), (28), (29), (30), (31), (32), who identified the various species with reasonable completeness based on the concept of specific and group factors and their role in determining antigenic relationships. Statistics prior to 1940 are of little value regarding type distribution, since antigenic structure study was not sufficiently advanced. Young (33) in a review of worldwide distribution recorded no data from Japan and Korea up to 1947, in which year Barksdale (34) reported types recognized in the Tokyo area. Sanders and Elrod (35) in 1950 reported that in Japanese and Americans in Tokyo, Sh. flexneri types were predominant, with 40% of the strains being type 2a. Sh. sonnei constituted 35% of the total encountered. The distribution resembled that seen in the continental United States except for the absence of Sh. flexneri 6, which was relatively common in America (36). A similar distribution of Shigella types was reported by Ewing and Gravatti in the Mediterranean Area (37), and by Cox and Wallace in American troops in India (38).

During 1951, a total of 12,074 specimens were examined, including 1,350 stool cultures. Exclusive of aerogenic enteric non-pathogens and Salmonellae, 4,308 strains identified as Shigella species were recovered. In addition, 536 anaerogenic strains were encountered, of which 430 are still under study as possible Shigellae. The cultures were obtained from American and UN troops, Japanese civilians, and from North Korean and Chinese POW's in prison camps at Pusan and Koje-do. This last group contributed most of the strains studied.

Cultures received on KIA slants were routinely plated on MacConkey's agar, to assure a pure culture isolate for identification, since many slants were contaminated, probably due to initial fishing from SS plates with transfer of suppressed but viable contaminants to the KIA slants. Typing direct from initial KIA slants was done only when volume of work demanded such emergency measures. Colonies from MacConkey's agar were fished to tryptose enrichment broth containing lactose and sucrose and incubated for four hours to provide an inoculum for KIA slant, motility agar, citrate agar, urease medium, and additional differentiating carbohydrates, as circumstances indicated. The double sugar broth, when not fermented, was used for indol test at 24 hours. All cultures confirmed as Shigella species were thus screened for utilization of glucose, lactose, sucrose, salicin, and citrate fermentation, for H_2S, gas, indol and urease production, and for motility. Mannite was included, where indicated, as a convenience in grouping strains. From 12% to 20% of cultures presenting possible significant KIA slants could be eliminated from further consideration on the basis of prompt lactose and/or sucrose fermentation in the double sugar broth. The presence of these carbohydrates did not affect the indol test since this procedure was not carried out when prompt fermentation occurred.

Serological typing was carried out by the slide agglutination technique, following essentially the method outlined by Wheeler (39) and by Ewing and Bruner (40). Confirmation by tube agglutination was carried out at frequent intervals and on questionable reactions, to affirm the correctness of the slide procedure. Typing sera employed were highly absorbed type-specific sera, prepared with pedigreed strains. Absorptions employed in preparing these sera were carried out as recommended by Kauffmann (41), Madsen (42), and by Edwards and Ewing (40). Comparisons with sera prepared by AMSGS were made at frequent intervals.

The polyvalent grouping sera were prepared by injection of pooled antigens and when necessary by pooling type specific sera. The mode of preparation assumed importance when strains were encountered which reacted in group but not in specific sera. This phenomenon raised a problem in identification of strains which is as yet unanswered.

Type distribution observed is shown in Table XX. Nomenclature is that recommended by the Enterobactericeae Subcommittee of the Nomenclature Committee of the International Association of Microbiologists.

Cultures from American military and civilian personnel in Japan yielded 18 species and types of Shigella, of which the predominant species, Sh. flexneri, was present in 77% of the cases. This incidence was exactly the same as that found by Elrod and Sanders in 1950 in the same population group and very near the average computed by Weil in 1947 on results reported throughout the world. The distribution of Sh. flexneri types during 1951 differed somewhat from previous studies. The predominant type was still Sh. flexneri 2a, present in 30.2% of cases. Sh. flexneri 3 and 4A were equally represented (17.8% and 17.3% respectively). However, 22 strains of Sh. flexneri 3 were obtained from a single shipboard outbreak.

Two of the Sachs-Large types of Sh. dysenteriae, type 4 (Q1167) and type 6 (Q454), have not previously been reported from the Far East, while the ubiquitous type 2 (ambigua) was represented. Two relatively uncommon boydii types, 2 and 4 were present. Sh. sonnei was less prominent than in former observations on Americans in Japan, and contributed but 8.2% of the isolates. The Alkalescens-Dispar group was represented by all 4 recognized types, A-D group 1 being predominant (31). The single "Y" strain recognized was a pure antigenic entity; circumstances of its isolation and subsequent study virtually precluded the possibility that it might be a culturally derived variant. The question of the validity of the "Y variant" will not be taken up at this point, but it constitutes the only convenient designation for this strain.

Table XX. Incidence and Distribution of Shigella Species and Types in Japan, Ryukyus and Korea, 1951

Species and Type	Americans in Japan		Americans in Ryukyus		Japanese		Korea (chiefly POW)	
	No.	%	No.	%	No.	%	No.	%
Sh. dysenteriae 1							24	.6
Sh. dysenteriae 2	7	3.2			2	.5	116	3.1
Sh. dysenteriae 4	1	.5					22	.6
Sh. dysenteriae 6	1	.5					13	.3
Sh. dysenteriae 7							1	.02
Sh. flexneri 1a	7	3.2	2	4.2	2	.5	182	4.9
Sh. flexneri 1b	9	4.1	4	8.5	18	4.8	11	.3
Sh. flexneri 2a	66	30.3	18	38.2	274	73.1	334	9.1
Sh. flexneri 2b	4	1.8			6	1.6	4	.1
Sh. flexneri 3	39	17.9	10	21.2	34	9.0	1028	28.0
Sh. flexneri 4a	38	17.4	1	2.1	9	2.4	1338	36.4
Sh. flexneri 5	5	2.3			1	.2	256	6.9
Sh. flexneri 6							11	.3
Sh. flexneri "X"							11	.3
Sh. flexneri "Y"	1	.5	1	2.1	2	.5	64	1.7
Sh. flexneri "VY"							1	.02
Sh. flexneri non-specific					1	.2	1	.02
Sh. boydii	2	.9					26	.7
Sh. boydii 2	2	.9	1	2.1				
Sh. boydii 4							1	.02
Sh. boydii 5							8	.2
Sh. sonnei I and II	18	8.2	9	19.1	26	6.9	43	1.1
A-D group 1	9	4.1					9	.2
A-D group 2	4	1.8	1	2.1			155	4.2
A-D group 3	1	.5					11	.3
A-D group 4	4	1.8					2	.05
Total	218		47		375		3672	

* Reacted in Sh. flexneri polyvalent serum but specific and group factors were not demonstrable

Examination of 47 strains received from Okinawa showed the predominant type to be Sh. flexneri 2a (38.3%) with Sh. flexneri 3 (21%) and Sh. sonnei (19%) of less frequent occurrence. This distribution paralleled that occurring in strains from Americans in Japan. The single Sh. flexneri 4a strain recovered on Okinawa fermented mannite, as did most American strains of this type. Types 1a and 1b, a variant "Y", a Sh. boydii, and one A-D group strain were also recognized.

The cultures from Japanese were obtained by weekly samplings of stool cultures from a large Tokyo contagious disease hospital. An average of 51.3 cultures per month were examined, of which 60% yielded Shigella on the basis of a single specimen. Of the 375 strains recovered, 92.5% were Sh. flexneri, with the majority (73.1%) type 2a. The distribution reflected the severe Shigellosis present in the local populace during the year. Sh. flexneri 3 with 9.0% of strains contributed a minor part of the flora. Sh. sonnei was present in only 6% of cases, which was a marked decrease from its incidence in previous years. Sh. ambigua was the only dysenteriae type present. In contrast to previous reports, no Sh. boydii types were found. Although this species is apparently nowhere abundant, it was surprising to find it absent in this group of Japanese patients. It is also deemed unusual that no Alkalescens-Dispar group cultures were recovered since these organisms have long been considered ubiquitous.

Elrod and Sanders (35) stated that a majority of V strains recovered in 1950 were VZ. In this series, 9 times as many 1b as 1a types were recovered. The striking preponderance of this mixed sub-type is of particular interest in contrast to its minor incidence elsewhere in the world (43). In a series of 15 strains, detailed attempts were made to recover a "Z" variant by plating single colonies, fishing 20 colonies and repeat plating. In no instance was evidence of this dissociation obtained.

Mention has been made of the frequency with which mannite-negative Sh. flexneri 4 strains are encountered in Japan. None of the flexneri 4 strains recovered from Japanese in Tokyo in 1951 fermented mannite. This finding is in striking contrast to that observed in Korea, North American countries and Western Europe. Since it was obvious that the mannite-negative strains might be Sh. rabaulensis or Sh. rio (? Sh. flexneri 4c) (44) such strains received careful attention, but no type 4c was found. In 1950, one Sh. rabaulensis strain was isolated in Tokyo. Type 4b is even more striking in its absence; all of the 1338 type 4 strains isolated were tested for group factor 6, in an effort to detect this type. No indication of its presence has yet been found, either in Japan or Korea.

The two "Y" variants were studied in detail from the initial plate as soon as their identity was detected. No specific type-reacting component could be detected, and the "Y" variant designation was considered to be valid on the basis of current taxonomy. The topic is discussed further below.

There was a large epidemic of Shigellosis in North Korean and Chinese POW's. Initial cultures were obtained and identified by the 8217th Mobile Medical Laboratory in Korea and at various times duplicate cultures were forwarded to this laboratory for corroborative study. Unidentified or anomalous strains were also submitted for identification. The Armed Forces Epidemic Disease Board Dysentery Research Unit took over a detailed study of epidemiology and comparative chemotherapy in the POW camp in May, 1951. Several complete series of cultures from these patients were received, together with additional lots of unidentified cultures which presented unclassifiable reactions. The total volume of cultures which were received for study (5,933) permitted a reasonably representative sampling of the flora of the epidemic.

A total of 25 types of Shigella have been identified from these Korean strains. Almost all currently accepted types are thus represented; only two Sh. dysenteriae (Sachs-Large 3 and 5) and four Sh. boydii (2, 3, 6, and 7) of those types and species listed by the Nomenclature Subcommittee, were missing.

Cultures received reflected the sequence of events during the epidemic of Shigellosis which, from January 1951 on, was present in the POW camp at Pusan. The predominant type initially observed was Sh. flexneri 4a, which in the annual total contributed 36.4% of isolates. After the first two months of observation, a sharp increase of type 3 occurred, and 28.0% of isolates were ultimately so classified. After 4 months the bulk of prisoners were transferred to the prison camp at Koje-do. The epidemic remained primarily one of Sh. flexneri 4a and 3, with 4a predominant. A sharp rise in the percentage of Sh. flexneri 5 occurred in July and August, with a corresponding relative drop in the two principal types. Type 5 decreased in frequency soon afterwards, its annual incidence being 6.9%. A rise in incidence of Sh. flexneri 1a and 2a also occurred in mid-summer, but took place more gradually, over a 2 month period. The latter reached 9% of total recoveries, while 1a was present in 4.9%. The low incidence of Sh. sonnei (1.1%) and of Sh. flexneri 6 (.3%) is not noteworthy. The latter two types are common in Japan, America, and Western Europe (45), (34), (43).

Sh. dysenteriae 2 was consistently present throughout the epidemic. However, no sharp peaks of incidence occurred, and the rate of 3.1% represented a level maintained throughout the year.

Alkalescens-Dispar group organisms have been classified with the Shigellae, although their taxonomic status is in doubt. A-D II (the Sh. tiete of de Assis) was the preponderant type of Alkalescens-Dispar organism in contrast to observations in North and South America that indicate it to be relatively uncommon. Since the primary isolation procedures were not performed in this laboratory the significance of fluctuation in recovery of A-D group organisms could not be evaluated. It is possible that strains were overlooked during the isolation process. There was a high incidence of thermolabile blocking antigens in these strains, and circumstances in the field did not always permit routine use of heated antigens. The initial serologic screening would thus lead to discard of such strains. It is of interest that at the end of the year the number of Alkalescens-Dispar strains received from Koje-do remained low, in contrast to the observation of Stuart, Rustigian and Corrigan (46) that late in an epidemic an increasing number of aberrant Alkalescens forms tend to appear.

The predominance of Sh. flexneri types 4a and 3 in the Korean POW epidemic was in marked contrast to the predominance of 2a in Japan. The actual incidence of type 4a was probably even greater than percentage results indicated, since in selection of cultures for confirmatory studies, strains of the most frequently encountered types might well be omitted.

The predominance of Sh. flexneri 1a over Sh. flexneri 1b observed in Korean strains was the reverse of that observed in cases in Japan and Okinawa and resembled the ratio of type 1a to 1b found by Ewing in the Mediterranean Area. Such divergence in relationship of these two types in two such adjacent geographic areas as Japan and Korea has apparently not been observed previously. In contrast, type 2a and 2b show a ratio of incidence consistent with that reported in other areas of the world. Type 2a was far more frequently present.

In January 1951, the first observation of the occurrence of Sh. flexneri 4a, phase B was noted. The type 4a strains recovered in Korea were very prone to dissociate, even on primary isolation, to the non-reversible B phase which was completely lacking in specific antigen. Twenty-three out of 48 Korean strains showed dissociation to individual phase B on first sub-culture after primary isolation. Conversely, the Sh. flexneri 4a strains recovered from Japanese cases did not show such spontaneous dissociation. When 10 strains of Sh. flexneri 4a from Japanese cases were plated and 20 colonies fished from each plate, no dissociation was noted. Serial transfer for over 90 passages was required in order to derive phase B from phase A in a single Japanese strain. This difference in lability of specific phase was the principal one noted between Japanese and Korean strains.

Seven laboratory technicians contracted Shigellosis during the year, with 2 repeat infections occurring. Four cases were in highly trained technicians working on

Shigella typing, while three others occurred in technicians assigned to other work in the same room with the enteric bacteriology section. The two most experienced individuals were infected in January and February, 1951. Other cases occurred in February, April, May, July, September, October, and December. A resume of cases is presented in Table XXI.

Table XXI. Laboratory Infections with Shigella flexneri

Case No.	Date of Onset	Infecting Type	Duties of Technician
1	January, 1951	Sh. flexneri 4a	Shigella typing
2	February, 1951	Sh. flexneri 4a	Shigella typing
3	February, 1951	Sh. flexneri 4a	Diagnostic antigen production
4	April, 1951	Sh. flexneri 4a	Diagnostic agglutination
5	May, 1951	Sh. flexneri 4a	T.B. diagnosis
6a	July, 1951	Sh. flexneri 4a	Shigella typing
6b	October, 1951	Sh. flexneri 5	Shigella typing
7a	September, 1951	Sh. flexneri var. newyork	Shigella typing
7b	December, 1951	Sh. flexneri 5	Shigella typing

The Shigella flexneri 4a strains from all these patients were mannite-positive, and from each isolate, phase B as well as phase A was promptly isolated. The phase B colonies were carefully checked for absence of specific antigen, and by all criteria were pure group phase cultures. These two characteristics typify the Korean strains as opposed to the Japanese variety.

The relative virulence of Shigella flexneri in man is such that in experimental infections 10^9 to 10^{10} organisms have been required to establish an infection (43). The cases occurring here were infected without observed accidental ingestion. Apparently these strains possessed exceptionally high virulence. Tests on case #1 established a MLD of 1×10^{-5} of an 18 hour broth culture for mice, indicating a relatively high degree of virulence.

All seven cases were hospitalized at onset of infection. All patients responded promptly to aureomycin therapy, except patient #2, who required chloromycetin for alleviation of symptoms. One patient continued to shed dysentery bacilli until treated with chloromycetin. Patient #1 was examined for appearance of agglutinin. The titers are shown in Table XXII. An antibody response to the group antigens of the organism was apparently present. It is distinctly probable that the infecting organism was one of those Shigella flexneri 4a strains which had been noted as being extremely labile in stability of phase. Agglutination studies revealed presence of anti-group factor agglutinin in other convalescent technicians. Unfortunately, paired specimens were not obtained in these cases. The possibility that the Korean strains were more prone to lose specific phase in vivo was suggested by these results, which were consistent with the serologic lability of the Korean strains.

Table XXI. Shigella flexneri Type Agglutinins
(reciprocal of titer)

Serum	1a	2a	2b	3	4a Phase A	4a Phase B	5	6	rabaulensis	X	Y
8 days (acute)	160	20	160	20	20	0				0	320
21 days (convalescent)	160	40	0	20	80	320	40	320	320	20	320

There were 430 strains which were biochemically acceptable as Shigella strains, but which could not be identified as members of any currently described species or type. The strains did not ferment lactose, sucrose, or salicin promptly. Urease was not formed, citrate was not utilized H_2S was not formed, and the organisms were non-motile. Glucose was fermented promptly without gas formation, and other carbohydrates

were fermented in varying patterns. Only U-P-negative strains were regarded as po-
tential Shigellae.

Characteristics of some of these strains included the following:

Group 1 (10 strains): Organisms were mannite negative, but fermented xy-
lose slowly. Reaction occurred in Sh. dysenteriae 1
and A-D-1 sera.

Group 2 (4 strains): Organisms showed a fermentative pattern possibly con-
sistent with Sh. flexneri. Unheated antigens reacted
in no antisera. Heated antigens reacted in Sh. flex-
neri 3 serum, but specific factors were apparently
not present.

Group 3 (15 strains): Strains did not ferment xylose, had a marked thermo-
labile blocking antigen, and a variable reaction in
A-D group sera. These strains also showed some cross
reactions with Sh. flexneri group sera.

Group 4 (40 strains): Organisms showed a positive indol reaction, but neg-
ative M-R and V-P tests and negative citrate utiliza-
tion. Mannite was not fermented. Positive group re-
actions were obtained in Sh. dysenteriae 1. Type
specific sera failed to permit identification.

The sensitivity to aureomycin, chloromycetin, streptomycin, terramycin, penicillin
and sulfadiazine of a series of recently isolated Shigella strains from Korea was deter-
mined by inoculation of fresh subcultures to agar plates containing graded amounts of
antibiotic. Results are summarized in Table XXIII. Terramycin and chloromycetin were
more effective than the other antibiotics. Sh. sonnei, as in previous studies, was the
least susceptible of the Shigellae. Aureomycin was moderately effective, streptomycin
was less effective, and penicillin was relatively ineffective. Sulfadiazine was inhib-
itory only in relatively large amounts.

Sensitivity results as compared to those recorded 10 months earlier on comparable
species and types from the same area showed in most instances no significant difference.
The strains tested in 1951 were all from a large group of patients among whom hundreds
of cases had been treated with the antibiotics listed. Although there was no way of
knowing that any given individual had been under any given previous treatment, no strik-
ingly resistant strains were encountered after this treatment period. The only marked
difference noted was that isolates obtained toward the end of the year were more sensi-
tive than those previously tested. This was most noticeable in the case of Sh. flexneri
1b, 2a, and 2b toward aureomycin.

INDUCED ANTIBIOTIC RESISTANCE OF CERTAIN SHIGELLA FLEXNERI TYPES: In vitro resist-
ance to freshly isolated strains of Shigella flexneri 2a, 3, and 4a in the specific phase
was induced. A method which permitted detection of a wide range of sensitivity was de-
vised, in which the antibiotic solution was placed in an agar well in a surface-seeded
plate. The diameter of the inhibition zone was correlated with a control zone derived
from growth of standard test strains, and the degree of sensitivity was thus determined
from a standard curve. Sensitivity determinations were made from broth cultures of strains
which had not been subcultured more than twice prior to this study. Four hour broth
cultures were used as inoculum. Antibiotic resistant strains were derived by subcultur-
ing growth from the inner margin of the inhibition zone of the sensitivity test plate,
thus obtaining colonies which were most resistant to the antibiotic at each passage.
Strains with a several-fold increase in resistance to each antibiotic were readily de-
rived from this procedure. These resistant strains were then subcultured serially on
plain agar to determine the duration of resistance. Resistance was not permanent, since
a gradual return of sensitivity was noted. However, after six transfers on plain agar,
some resistance still persisted. Results are shown in Table XXIV.

Table XXIII. Sensitivity Of Shigella To Antibiotics

Species and Type	Aureomycin mcg/cc.	No.*	Chloromycetin mcg/cc.	No.	Minimal Inhibitory Concentration Terramycin mcg/cc.	No.*	Streptomycin mcg/cc.	No.*	Penicillin u/cc.	No.*	Sulfadiazine mg/cc.	No.*
Sh. dysenteriae 1	2.5 10	2	2.5 5	2	1.25 2.5	2	10	2	20	2	20 40	2
Sh. dysenteriae 2	2.5	1	2.5	1	2.5	1	10	1	20	1	10	1
Sh. flexneri 1a	5 10	2	2.5 5	2	1.25 2.5	2	5 20	2	10 20	2	20 40	2
Sh. flexneri 1b	5 10	4	2.5 5	4	1.25	4	10	4	10 40	4	20 40	4
Sh. flexneri 2a	2.5 10	6	2.5 5	2	2.5 10	9	10 20	6	10 20	5	40	5
Sh. flexneri 2b	2.5	1	2.5	1	1.25	1	10	1	40	1	40	1
Sh. flexneri 3	10	6	2.5 5	3	5 10	9	10	6	10 20	6	40	1
Sh. flexneri 4a	10 20	6	2.5 10	28	5 10	33	5 10	6	20 40	5	20 40	6
Sh. flexneri 5	5	1	5	1	2.5	1	10	1	40	1	20	1
Sh. flexneri 6	10	1	2.5	1	20	1	10	1	40	1	20	1
Sh. boydii 1	10	1	5	1	10	1	10	1	40	1	20	1
Sh. sonnei	20 40	2	10	2	20	1	20	1	40 80	2	40	1
A-D 1	5	1	2.5	1	10	1	10	1	20	1	40	1
Total		34		49		66		33		32		27

* Number of strains tested

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Minimum Inhibiting Concentration On Initial Test

Culture	Chloromycetin γ/cc	Terramycin γ/cc	Aureomycin γ/cc	Streptomycin γ/cc
Sh. flexneri 2a (4 strains)				
Initial	5	2.5	2.5	10
4th subculture*	40	40	80	80
8th subculture**	10	10	10	20
Sh. flexneri 3 (4 strains)				
Initial	5	10	10	10
4th subculture*	80	40	80	80
8th subculture**	10	10	10	10
Sh. flexneri 4a (5 strains)				
Initial	5	10	10	10
4th subculture*	40	40	80	80
8th subculture**	40	20	40	40

* 4th culture in medium containing antibiotic
** above plus 4 subcultures in agar without antibiotic

Table XXV. Antibiotic Sensitivity of Sh. flexneri 4a Phase A and B

Strain No.	Terramycin γ/cc		Chloromycetin γ/cc	
	Phase A	Phase B	Phase A	Phase B
621	10	20	2.5	5.0
627	10	20	2.5	5.0
674	5	20	2.5	5.0
633	10	20	2.5	5.0
638	10	20	2.5	5.0
649	10	20	5.0	10
656	5	10	2.5	5.0
651	5	10	2.5	5.0
644	10	20	2.5	5.0
711	10	20	2.5	5.0
715	5	10	2.5	5.0

A comparison of sensitivity of phase A and phase B of Sh. flexneri 4a to terramycin
and chloromycetin was made using strains which were bi-phasic on primary isolation (a
characteristic noted thus far only in strains from Korea). Table XXV shows results of
comparative tests. The difference in sensitivity between the two phases is slight but
consistent. Observations with streptomycin and aureomycin yielded similar results.

A strain of Sh. flexneri 4a (#1301) which had shown no tendency toward dissocia-
tion was passed by serial culture in the presence of antibiotic using the agar-well
method. The culture was initially pure phase A. The phase of the resulting subcul-
tures was determined. Control transfers was made on plain agar as each antibiotic
transfer was made.

The immediate effect of chloromycetin, terramycin, and streptomycin was to cause
a reversion of the culture to the non-specific phase B. Aureomycin did not produce this
change at all. The findings are shown in Table XXVI.

Table XXVI. Effect of Antibiotic on Phase of <u>Sh</u>. <u>flexneri</u> 4a

Antiserum	Organism				
			3 passages thru antibiotic		
	Aureomycin	Chloromycetin	Terramycin	Streptomycin	Control
4a - phase A	4✓	2✓	-	-	4✓
4a - phase B	-	4✓	4✓	4✓	-
			5 passages thru antibiotic		
Phase A	4✓	-	-	-	4✓
Phase B	-	4✓	4✓	4✓	-

Exposure of the induced phase B strains to aureomycin was carried out in an attempt to induce reversal to phase A. Three aureomycin passages of each strain were carried out following the fifth antibiotic passage which was shown in Table XXVI. No alteration occurred: the phase B was still phase B, and the strain passed a total of 7 times through aureomycin was still phase A. In turn, the phase A strain conditioned by aureomycin was passed three times through chloromycetin, terramycin, and streptomycin. Surprisingly, it remained phase A. Tables XXVII and XXVIII summarize the phase manipulations described. Although much more work will be required before the significance of these findings can be evaluated this phenomenon opens up a whole new field of study of <u>Shigella</u> phase relationships.

Table XXVII. Passage of Antibiotic - Modified Phase Through Aureomycin

Antiserum	Organism				
	Aureomycin A - 7	Chloromycetin $C_5 - A_3$*	Terramycin $T_5 - A_3$	Streptomycin $S_5 - A_3$	Control
4a A	4✓	-	-	-	4✓
4a B	-	4✓	4✓	4✓	-

* $C_5 - A_3$: 5 passages through chloromycetin plus 3 passages
through aureomycin. Similar treatment for $T_5 - A_3$
and $S_5 - A_3$

Table XXVIII. Passage of Aureomycin-Treated Phase A Through Other Antibiotics

Antiserum	Organism			
	$A_7 - C_3$*	$A_7 - T_3$	$A_7 - S_3$	Control
4a A	4✓	4✓	4✓	4✓
4a B	-	-	-	-

* $A_7 - C_3$: indicates strain passed 7 times through aureomycin and then three times through chloromycetin.
Similar treatment for $A_7 - T_3$ and $A_7 - S_3$.

SALMONELLA TYPES IN JAPAN AND KOREA, 1951: During 1951, the Department of Bacteriology identified 937 Salmonella strains. With the exception of the human-adapted strains recovered in blood cultures, (chiefly from North Korean POW's and from some patients in Japan), the strains were from stool cultures. In Korea the dysentery epidemic in POW's was accompanied by an epidemic of Salmonellosis, in which types <u>paratyphi</u> A and <u>enteritidis</u>, <u>choleraesuis</u>, <u>paratyphi</u> B and <u>paratyphi</u> C predominated.

Stools and rectal swabs were plated directly on SS agar, and significant colonies
were fished to Kligler's Iron Agar. Kauffman's modification of tetrathionate enrich-
ment broth was also employed, and greatly increased the recovery of Salmonellae in Korea.
Biochemical screening criteria included lactose, sucrose, and salicin fermentation, and
indol and urease formation. Urease and indol positive organisms were discarded. Motil-
ity tests with semi-solid agar and phase separation according to the method of White
were employed. Potential Salmonella strains were first tested with Salmonella polyvalent
"O" serum. Positive-reacting strains were tested with definitive typing procedures of
Kauffmann and of Edwards and Bruner (45).

A resume of findings is presented in Table XXIX. Of 7 strains recovered from 616
cultures (1.1%) from patients in a Japanese contagious disease hospital, four were Sal. ty
phi and three were Sal. paratyphi A.

In Japan the total number of Salmonella recovered was low with Sal. paratyphi A, par-
atyphi B, paratyphi C and typhi predominating. The human-adapted, systemic strains ob-
viously represent the principal part of the restricted Salmonella picture in this popula-
tion group. The "food-poisoning" types are conspicuous by their absence. Of particular
interest were the 4 strains of Sal. abortus-bovis recovered in Japan. This organism was
not recorded as a human pathogen by Bergey (6th Ed.), Topley and Wilson (3rd Ed.,) or
others (47), (48). This is one of the few Salmonella types said to liquify gelatin;
however, these strains did not. The strains were recovered during January and February
of 1951, from two soldiers, a WAC and a civilian. Each was examined in reference to a
complaint of ill-defined abdominal distress and irregular diarrhea. There appeared to be
no epidemiologic connection between the cases.

Sal. typhimurium, the type predominant in Europe, (49), (50), and frequent in North
and South America (51) had an unexpectedly low incidence in Japan while paratyphi C was
surprisingly numerous. The remaining five scattered strains of group C and C were not
unusual, except for Sal. mission which had previously been reported only from Texas.
Typhoid fever occurred sporadically in Japanese and Americans. Strains were equally di-
vided among Vi positive and Vi negative. The cases of Sal. enteritidis were all systemic
enteric fever infections. All Salmonella strains in Japan were obtained from cases of
systemic or enteric infections. No carrier strains were knowingly included.

In the Korean POW population, the situation was entirely different from that in
Japan. During the early months of the year the acute epidemic was caused primarily by
Sal. paratyphi A. Numerous perforations of the ileum from the excised tissue, of which
some strains of paratyphi A were recovered, were attributed to this organism. The in-
fection rate remained high throughout the year, but the intestinal perforations ceased
when TAB vaccination was carried out on all POW.

Sal. paratyphi B showed a four-fold rise in incidence in the latter half of the
year, to a rate comparable to that found in Japan during the same period. Most strains
were recovered from the blood. The incidence of Sal. typhimurium was unexpectedly low,
reaching at most 2.2%, Sal. paratyphi C, Sal. choleraesuis, and Sal. typhisuis were
numerically important throughout the year. This serologically closely related group of
organisms has long constituted a biologically confusing picture, even after the types
were differentiated by White in 1926 (52), on the basis of biochemical reactions. The
classic picture of this group may be represented as follows:

Organism	Phase	H$_2$S	Arabinose	Trehalose
Sal. paratyphi C hirschfeldii	Diphasic	≠	≠	≠
Sal. choleraesuis american	Diphasic	–	–	–
Sal. choleraesuis kunzendorf	Monophasic Non-specific	≠	–	–
Sal. typhisuis	Diphasic or monophasic (non-specific)	–	≠	≠

Table XXIX. <u>Salmonella</u> Types In Japan and Korea

Group	Type	Tokyo (Americans and Japanese)		Korea POW's Jan - April (Pusan)		Korea POW's May - Dec. (Kojedo)	
		No.	%	No.	%	No.	%
A	paratyphi A	6	13.6	235	36.8	84	32.9
B	paratyphi B	7	15.9	21	3.2	34	13.4
	abortus-bovis	4	9.1				
	chester			1	0.15		
	san-diego	1	2.2	1	0.15		
	typhimurium	2	4.5	14	2.2	5	1.9
C_1	paratyphi C	5	11.3	65	10.2	33	12.9
	choleraesuis			39	6.1		
	typhisuis			2	0.31		
	mission			1	0.15		
	montevideo	1	2.2	5	0.8	13	5.1
	thompson					1	0.4
	potsdam					2	0.7
	oranienburg	1	2.2	17	2.6	10	3.9
	bareilly			1	0.15	1	0.4
	tennessee			2	0.31		
C_2	muenchen	1	2.2				
	newport	1	2.2				
	kottbus					1	0.4
	bonariensis	1	2.2				
D	typhi	8	18.2	51	8.0	21	8.2
	enteritidis	6	13.6	113	17.7	41	16.1
	blegdam			70	11.0	7	2.7
E_1	give					1	0.4
E_2	senftenberg					1	0.4
	Total	44		638		255	

Bruner pointed out the peculiar phenomenon of the gradual decrease in incidence of the diphasic, H_2S-negative type from the United States during the 1925 - 1947 period, and its replacement by the <u>kunzendorf</u> type. A detailed study of 6 strains of <u>Sal</u>. <u>paratyphi</u> C and of 39 <u>choleraesuis</u> from Pusan was made, after discrepancies in identification were noted in a series of cultures. The variations in biochemical behavior are summarized in Table XXX. The classic differentiation of <u>Sal</u>. <u>paratyphi</u> C as diphasic, H_2S positive and <u>trehalose</u> and <u>arabinose</u> fermenting was complicated by one H_2S negative strain (distinguishable from <u>typhisuis</u> only by presence of Vi antigen), one trehalose negative strain (which might as readily be regarded as an <u>arabinose</u> positive <u>choleraesuis</u>), and by four monophasic strains (which in this respect resembled some naturally occurring <u>Sal</u>. <u>choleraesuis</u> <u>var</u>. <u>kunzendorf</u> strains).

The <u>Sal</u>. <u>choleraesuis</u> strains showed some divergence from the supposed relationship between phase and H_2S formation. Among the 23 <u>kunzendorf</u> strains, the majority were non-specific in phase, but some were in the specific phase. This phenomenon was noted by Edwards (47) but has been assumed to be rather uncommon. It may be regarded as a natural phase reversal. One monophasic, H_2S-negative <u>kunzendorf</u> type strain appeared to represent a further progression in variation. The significance

Table XXX. Comparative Reactions of Sal. paratyphi C and Sal. choleraesuis

Strain	No. of Strains	Phase	H$_2$S	Trehalose	Arabinose
Sal. paratyphi C	59 (4 Vi +)	Diphasic	+	+	+
Sal. paratyphi C	1 (vi +)	Diphasic	-	+	+
Sal. paratyphi C	1	Diphasic	+	-	+
Sal. paratyphi C	4 (1 Vi +)	Monophasic (specific)	+	+	+
Sal. choleraesuis (american)	10	Diphasic	+	-	-
Sal. choleraesuis (american)	5	Diphasic	-	-	-
Sal. choleraesuis (kunzendorf)	23	Monophasic (chiefly non-specific)	+	-	-
Sal. choleraesuis (kunzendorf)	1	Monophasic	-	-	-
Sal. typhisuis	2	Diphasic	-*	+ (3 da.)	+ (2 da.)

* An H S forming variant could be derived from these strains

of type differentiation is greatly lessened by the observation of such intermediates.

The Sal. typhisuis strains were originally classified by the submitting laboratory as kunzendorf strains. The failure to form H$_2$S and tardy fermentation of trehalose and arabinose indicated their identify. Plating and checking the identity of single colony isolates confirmed the identification as Sal. typhisuis. However, an H$_2$S variant could be recovered in 10% of the colonies. This variant resembled the kunzendorf pattern except for the trehalose and arabinose fermentation. The variant was unstable and Sal. typhisuis forms could be derived from subcultures. The specific phase in the typhisuis strain was weak but positive. These two strains were obtained from blood and tissue at autopsy, suggesting that typhisuis may be on occasion pathogenic for man. A study of 20 additional strains of choleraesuis failed to reveal other examples of this variant form.

Sal. montevideo and oranienburg were the next most common types of the group C strains. Both were recovered from stool and blood stream. The former was more common at Koje-do than in Pusan. Eight percent of the Salmonella were Sal. typhosa. Of these, 31 were Vi-positive, 14 Vi-negative, 3 mixed Vi-positive and negative, and one showed a V-W phase variation. Sal. enteritidis was the principal group D strain found, and second only to Sal. paratyphi A in overall incidence. Most of the enteritidis strains were recovered in blood cultures, and their pathogenicity was apparently high. Sal. blegdam was overlooked for several months, due to a defective "q" factor serum. Its identity was recognized on re-examination with a freshly prepared factor "q". The incidence of Sal. blegdam declined markedly after the POW's were moved to Koje-do. This appeared to be the first reported large-scale infection with this type, which was never observed in the U.S. over a 13 year period, (47).

EHF AND LEPTOSPIROSIS: When Epidemic Hemorrhagic Fever first occurred in United Nations troops in Korea, it was suspected that the disease was due to Leptospirosis. Intensive efforts, described below, to confirm this diagnosis were fruitless. It is believed that Leptospirosis was excluded as a possible cause of EHF.

Blood from 45 patients and catheterized urine from 30 patients were cultured on Korthof's medium at 25° and 32°C. Material from 30 cases was cultured on Schuffner's

medium and from 28 cases on Fletcher's medium. In 20 of these 30 cases multiple blood and urine cultures were made while in the remainder only specimens obtained during the fourth to the tenth day of the disease were used. A total of 375 cultures were run on 160 blood samples and 186 cultures on 65 urine samples. All of these cultures were negative for Leptospirae although 23 blood cultures showed bacterial contamination.

Liver, kidney, lung and spleen obtained at autopsy from four fatal cases were also cultured as above. The specimens had been shipped in the frozen state or at 4°C. This had been shown not to affect the survival of Leptospirae. Only small amounts were inoculated on the media. In three of these cases cultures had also been taken prior to death. All cultures were negative.

Material from 42 patients, including 6 who died and were autopsied, was injected into animals. Guinea pigs weighing approximately 175 grams were inoculated intraperitoneally with 1 to 5 cc of urine, 0.5 to 1 cc of heparinized blood, titurated blood clot and serum or 1 cc of ground human liver, kidney, spleen or lung. Hamsters weighing 25 to 30 grams received 0.5 cc of blood, 1 to 5 cc of urine or 0.3 cc of ground tissue. Heart blood was drawn on the fifth to sixth day from guinea pigs and on the fourth day from hamsters and whenever a rise in the daily temperature occurred. If the temperature rise occurred after the fourth day, the animal kidney, liver, heart's blood, peritoneal fluid and urine were cultured, a 20% emulsion of ground liver and kidney was injected into young guinea pigs and hamsters and tissues were studied for histopathological changes. These second passage animals were sacrificed at 10 to 14 days or sooner if symptoms appeared and a similar third and fourth series of cultures and passages were made. One group of 20 animals was observed for 21 days prior to sacrifice. At each step, tissue emulsions, blood, and urine were examined by darkfield illumination for the presence of Leptospirae. Antibiotics were administered to some of the animals when cultures of animal material on blood agar plates indicated that symptoms were due to bacterial infection. Blood and urine from 7 patients was injected into white mice because these were the only animals available at the Korean installations. Material from these mice was later passed into guinea pigs and hamsters.

The work done with animals is summarized in Table XXXI. No Leptospirae were recovered from any of these animals. Twenty of the guinea pigs and ten hamsters developed intercurrent bacterial infections. There were 10 guinea pigs which died 7 to 10 days after inoculation. Passage of material from three of these to fresh guinea pigs caused death in 5 to 12 days. No Leptospirae or bacteria were recovered from these animals. Some of the tissues were submitted to the virus department. The unknown lethal agent was lost when a Salmonella infection appeared in the inoculated animals.

Table XXXI. Attempts to Isolate Leptospirae in 286 Animals with
Material from 42 Cases of EHF

Human Material Inoculated	Animal	No. of Cases	No. of Animals	No. of Passages in Animals (cases)				Cultures from animals
				1	2	3	4	
Blood	Guinea pig	28	70	6	8	8	6	326
Blood	Hamster	19	52	2	5	8	4	160
Urine	Guinea pig	30	65	11	8	8	3	274
Urine	Hamster	19	47	7	7	4	1	241
Liver and Kidney	Guinea pig	6	17	0	3	2	1	89
Liver and Kidney	Hamster	6	18	0	2	2	2	94
Lung and Spleen	Guinea pig	3	9	0	1	1	1	39
Lung and Spleen	Hamster	3	8	0	1	2	0	43

The agglutination-lysis test of Schuffner-Mochtar (53) with 4 strains of living Leptospirae was used on 201 sera from patients with EHF and on 10 sera using 12

strains of Leptospirae. The serum was inactivated (56°C. for 30 minutes) and diluted serially from 1:50 to 1:6400. A four to five day old Korthof's broth culture of Leptospirae checked for the presence of non-specific clumping was used as the antigen. The serum and antigen were incubated at 30°C. for 3 hours and examined under darkfield illumination. At first, oil immersion preparations were used but later power examination (10 X objective and 15 X occular) without using a cover slip was used. This technique expedited reading the test without adversely affecting accuracy. A test was read as positive when clumps of twisted Leptospirae were seen or when granular masses of degenerating organisms and a decrease in the number of organisms when compared to the control was found. The end point of the agglutination reaction was indicated by the presence of numerous immobilized Leptospirae.

Initially, 4 laboratory strains of Leptospirae obtained from the Japanese NIH were used. The identity of these strains was checked with standard type strains from the AMSGS as shown in Table XXXII.

Table XXXII. Identification of 4 Strains of Leptospirae Used for the Agglutination-Lysis Test

NIH	AMSGS
L. hebdomadis B	L. hebdomadis B
L. australis A	L. australis A (ballico)
L. hebdomadis A	L. autumnalis (closely related to akiyami A)
L. icterohemorrhagiae	L. icterohemorrhagiae

In performing the test a screening level of 1:50 was used. Of the 201 sera examined, 63 (31%) gave positive reactions at titers of 1:50 to 1:200. Of these 63 sera, 17 reacted with L. icterohemorrhagiae, 18 with L. hebdomadis A, 4 with L. hebdomadis B, and 24 with L. australis A. Titers at this level do not indicate the presence of active or recent infection. The following 12 species of Leptospirae were made available by the AMSGS.

L. icterohemorrhagiae Wijneberg
L. canicola (Ruebusch)
L. bataviae (Swart van Tienem)
L. pomona
L. australis ballico KM
L. autumnalis (akiyami A Pasteur Institute)
L. grippo-typhosa (Andaman CH 31)
L. ballum (Pasteur Institute)
L. hebdomadis A (406 MGL)
L. pyrogenes salinem (Pasteur Institute)
L. hebdomadis (Pasteur Institute)
L. mitis Johnson (National Institutes of Health)

Ten paired sera were tested using all of these strains and a screening dilution of 1:100. Six sera which had shown the low titer reactions described above were included in this group of 10 sera. All sera, both acute and convalescent, were negative.

The complement fixation test, a more rapid screening test used successfully at the AMSGS (54), was employed on 101 sera. Three sonic disintegrated strains, L. icterohemorrhagiae W., L. bataviae SvT and L. pomona had been shown to suffice for screening antibody response to all species of Leptospirae currently recognized. Seventy-nine samples were negative, 18 were anticomplementary, three gave a 2+ reaction and one a 3+ reaction, with undiluted serum. The four remaining sera were negative when tested for agglutinins with all 12 antigens at 1:50 dilution.

Equine sera were obtained as part of a program of search for intermediate host or vectors. Of six horse sera examined by complement fixation test, one was anticomplementary, three gave a 2+ reaction, one a 3+ and one a 4+ reaction in undiluted serum.

Agglutinins were demonstrated in four of the five sera at 1:50 but none at 1:200. Of these, one reacted with L. pyrogenes, and L. hebdomadis A; one reacted with L. canicola, L. bataviae, L. akiyami A., and L. hebdomadis A; and one reacted with L. pomona, L. akiyami A., L. ballum, L. hebdomadis A, and L. canicola. The significance of these low level titers in equine sera is not known. Yager et al (55) found 37% of 86 horses to have positive titers below 1:300, while an additional 12% showed titers of over 1:300 to L. pomona.

ISOLATION OF LEPTOSPIRA FROM KOREAN RODENTS: A series of rodents, identified as Apodemus agrarius, were live-trapped in Korea, in a center of incidence of EHF. The animals were shipped to Tokyo by air, and were sacrificed with chloroform. After ectoparasites had been collected, each group of animals was dissected aseptically. One hundred rodents, in 28 lots, were examined. A portion of liver and kidney from each animal was removed, pooled, and ground with sand in a sterile mortar to make a 10% suspension. Multiple tubes of Korthofs' and Fletcher's media (56), (57), (58), were inoculated using .05 cc of the ground suspension per tube. Darkfield examinations of the tissue brie were made. One to 2 cc. of emulsion was injected into 175 gram guinea pigs. Tissue blocks were fixed for histologic examination.

Guinea pigs were observed for 10 days and then treated as described in the section on isolation attempts including carrying to a third passage. Temperatures of all inoculated guinea pigs were taken daily. However, this did not aid in detecting the presence of infection, although the procedure is widely recommended. Cultures for Leptospirae were routinely examined at approximately 7, 14, and 28 days. Early in the study, they were examined at 24 hours. Later, it was concluded that adequate assurance of detection of spirochaetes would be obtained with examination at 14 and 28 days. Whenever bacterial contamination was noted, 0.5 cc. of the culture concerned was injected intraperitoneally into a 30 gram hamster from which the heart blood was cultured at 4 days. This technique worked well with all positive cultures.

Two strains of Leptospira were recovered. Strain #60-A was obtained from a pool of three specimens of A. agrarius trapped near Kumju-ri, Korea, on 26 August 1951. No gross pathologic changes were noted in the animals at autopsy. Korthofs' medium, inoculated with ground rodent tissue, was examined at 9 days, and Leptospirae were found to be present. The inoculated guinea pig was sacrificed at 10 days. Leptospira were seen in direct darkfield examination of the kidney-liver emulsion. The initial culture was inoculated into hamsters and guinea pigs. Hamsters yielded positive cultures from heart blood at 4 days. The organism was recovered from guinea pigs in culture at 6 to 10 days. This process was readily repeated with all subsequent inoculations. Guinea pigs were killed by this strain on initial inoculation but hamsters survived the first passage. After passage through guinea pigs and hamsters the strain became virulent for hamsters.

Strain #48 was obtained from a pool of 4 specimens of A. agrarius, trapped at Kohangchon, Korea, on 22 August 1951. Culture of tissue in all three media were negative. The initial guinea pig inoculated with tissue died at 11 days, without significant gross pathologic findings and tissue emulsion was negative for spirochaetes. The second passage guinea pig died at 5 days. Again, no significant findings were noted and no spirochaetes were seen in darkfield examination of tissue emulsion. On passage to a third guinea pig, the animal was moribund at 3 days. No organisms were seen in tissues or recovered in culture from this animal. Korthofs' culture from the initial guinea pig tissue was negative through 14 days but leptospira were seen at 28 days. Cultures were markedly virulent and the strain was readily re-established in culture from animals inoculated with it. Both hamsters and guinea pigs were killed by inoculation of the initial positive culture. The autopsy and histological changes in these animals are described in the Pathology portion of this report.

In culture, strain #48 was more fastidious than strain #60-A and required several transplants before it was securely established. Fletcher's medium was more satisfactory than the others for both strains in maintaining heavy concentrations of viable organisms over periods of at least a month, both at room temperature and at 32°C.

The two strains were tested against hyperimmune sera for the 12 species supplied by AMSGS, the results of which are shown in Table XXXIII. Both strains reacted principally with L. icterohemorrhagiae and L. canicola. Weak reactions in L. bataviae, L. akiyami A, and L. salinem appeared with strain #60-A while strain #48 reacted weakly only with L. akiyami A.

Antisera using formalin-inactivated strains #60-A and #48 were prepared in rabbits. Cross reactions were carried out to show the extent of serologic relationship of these strains with L. icterohemorrhagiae, L. canicola, L. akiyami and with each other. The results are shown in Table XXXIV.

Table XXXIII. Serologic Reaction of Leptospira #60-A and #48

Antiserum Titer	Strain #60-A			Strain #48		
	1:100	1:400	1:800	1:100	1:400	1:800
L. icterohemorrhagiae W	2/	2/	2/	2/	3/	2/
L. canicola R	2/	2/	2/	2/	2/	1/
L. bataviae SVT	/	/	-	-	-	-
L. pomona	-	-	-	-	-	-
L. australis ballico	-	-	-	-	-	-
L. akiyami A PI (autumnalis)	/	/	⫽	/	⫽	-
L. grippotyphosa ACH 31	-	-	-	-	-	-
L. ballum PI	-	-	-	-	-	-
L. hebdomadis A	-	-	-	-	-	-
L. pyrogenes salinem	⫽	⫽	-	-	-	-
L. hebdomadis PI	-	-	-	-	-	-
L. mitis J.	-	-	-	-	-	-
Antigen-normal serum	-			-		
Antigen-saline	-			-		

Table XXXIV. Serologic Reaction of Leptospira #60-A and #48

Antigen	Icterohemorrhagiae	#60-A	#48	Canicola	Akiyami A	Control
L. icterohemorrhagiae	102400	1600	3200	1600	800	-
#60-A	102400	12800	12800	3200	800	-
#48	102400	12800	12800	6400	800	-
L. canicola	1600	800	800	102400	200	-
L. akiyami A	200	100	100	100	12800	-

In terms of proportion of hemologous to heterologous titers, strain #60-A reacted to titer with L. icterohemorrhagiae serum. The homologous L. icterohemorrhagiae reaction was, however, more intense than was the reaction of #60-A at 1:102400 dilution of serum. The converse reaction of L. icterohemorrhagiae in #60-A serum did not reach the homologous serum titer, which was 8-fold the heterologous reacting titer. Apparently strain #60-A possessed not only a complete L. icterohemorrhagiae antigen, but additional distinctive antigenic factors as well. In cross-reaction with L. canicola, strain #60-A exhibited a common factor, which produced an almost identical degree of reciprocal reaction between the respective organisms and their antisera. The cross-reaction of strain #60-A with L. akaiyami A was considered to be of a minor order. It paralleled the reaction of L. icterohemorrhagiae with L. akiyami A.

Strain #48 reacted to homologous titer with L. icterohemorrhagiae antiserum. L. icterohemorrhagiae in #48 antiserum did not reach the homologous titer, which was 4-fold the heterologous reaction. This reaction pattern resembled that occurring between #60-A and L. icterohemorrhagiae. Cross-reaction of #48 with L. canicola and with L. akiyami A resembled those observed with Strain #60-A.

The two Korean strains isolated appear to be the first reported from that country (59). On the basis of cross-reactions, they are believed to be closely related to L. icterohemorrhagiae, but there is serologic evidence of presence of a distinctive antigenic component. The absence of icterogenic changes produced by strain #48 and the moderate icterus produced by #60-A in guinea pigs constitute an additional point of discrepancy between L. icterohemorrhagiae and strains #60-A and #48. Further investigation will be carried out.

PROCESSING OF EHF CONVALESCENT SERUM: In late November, 1951 the Bacteriology Department was requested to process convalescent serum from EHF cases. The following procedures were employed:

a. Blood was collected in 250 cc. amounts in sterile transfusion sets which had been drained of anticoagulant. The bottles were kept at room temperature for 4 hours in a slanted position. The blood was then held for 44 hours at 4°C - 8°C in a mechanical refrigerator. This resulted in maximum clot retraction and serum yield.

b. Serum was withdrawn from bottles and subsequent manipulations were carried out in a room set aside, away from the bacteriology laboratory, for this purpose. The serum processing room was sprayed and wiped down with 5% cresol, prior to use, and ultraviolet air sterilization was employed. A glass hood and steam from an electric sterilizer were used during transfer and bottling operations to obtain a vapor-saturated environment to reduce the bacterial content of the air.

c. Equipment for transferring serum aseptically in a closed system consisted of the following items:

(1) 250 cc. centrifuge bottles and solid rubber stoppers each partially perforated by a drill to leave four one-fourth inch diaphragm-covered areas for ready penetration by an aspirator needle.

(2) Air-way consisting of #18 hypodermic needle attached to a 3 inch cotton-filled glass tube.

(3) 10 inch, 13 gauge plasma aspirating needles.

(4) Two or four liter pooling bottles with outlets at base, and solid rubber stoppers, partially perforated as in a. above. The outlet passage included a nylon transfusion-type filter and a filling shield.

(5) Pyrogen-free rubber tubing for all connections.

d. Serum processing was carried out as follows:

(1) Bottles of blood with contracted clots were transferred to the serum processing room. Sterile centrifuge bottles were fitted with sterile semi-perforated stoppers under the hood, using flame and sterile forceps for handling. The hood was continuously steamed during operation.

(2) The top of the blood bottle was sterilized with iodine and 70% alcohol (ignited). A sterile airway was inserted into one part of the stopper and then the #13 plasma aspirator needle was thrust through the stopper to the bottom of the bottle. The sterile aspirator assembled with a rubber tube was connected to an 18 gauge needle which was thrust through the chemically sterilized diaphragm stopper of the centrifuge bottle. A sterile airway was inserted in this stopper, prior to pumping serum into the bottle.

(3) An atomizer bulb was used to pump serum over into the centrifuge bottle, air being forced into the blood bottle through the sterile airway. The usual yield of serum (which contained some cells) from 500 cc. of blood was 180 to 200 cc.

(4) Bottles were centrifuged at 2000 RPM for 30 minutes. The rubber stoppers were covered with sterile gauze, fastened in place with rubber bands, during this operation.

(5) After iodine-alochol flame sterilization, a sterile aspirator needle was used to enter the stopper of the centrifuge bottle and, with positive pressure applied through a sterile airway, the serum was forced into the pooling bottle through a diaphragm-perforated stopper. Unavoidable loss demanded by avoidance of residual cell surface reduced the serum yield to 180 cc. to 190 cc. per donor. Serum was harvested in pools from 5 donors each.

(6) Pooled serum was drawn off through a transfusion filter into 100 cc. gauze-plugged, paper-capped serum bottles. The operation was carried out under the steamed hood, over sterile, cresol-wet towels. Each time the bottle was opened, the mouth was flamed. Sterility checks on the pool were inoculated into broth tubes at the beginning and end of each pool operation. One hundred cc. was arbitrarily designated as 1 unit of serum. No preservative was added.

(7) The gauze plugs were replaced as each bottle was filled, until the serum pool was distributed. Then rubber-mantled stoppers were inserted, using flame and sterile precautions, including sterile rubber gloves.

e. Sterility Tests:

(1) "NIH Minimum Requirements for ..... Normal Human Serum, 3rd revision, 10 January 1946" was followed. One week after the serum was bottled, 20 percent of the units from pools which were sterile at the time of bottling were tested by removing 5 cc. from each bottle. When the sterility test on an initial pool was positive, as happened in two instances, each bottle in that pool was checked. Pyrogen tests on one-third of the pools and a toxicity test on each pool, (injection of serum i.p. into young guinea pigs) were also run.

f. Storage of Serum:

(1) The serum was stored at $4°C$. for the prescribed minimum period of 28 days. Then, if all safety tests were satisfactory, permanent labels were attached, the serum bottles were frozen in a dry ice chest, and then stored at $4° - 10°$ F, in a mechanical refrigerator. Over 100 units of convalescent EHF serum and 90 units of normal serum were prepared.

DEPARTMENT OF CHEMISTRY

The Department of Chemistry, consisting of functional sections of Clinical Chemistry, Food Chemistry, Toxicology, Water Analysis, Allergy Investigation, and Miscellaneous Analysis, serves as an analytical chemistry laboratory for the Far East Command. The variety of requests submitted by the various military organizations of the Far East Command has been broad, and total work load has increased during the year 1951.

Table I. Chemical Examinations

Section	Specimens Submitted			Determinations Completed		
	1950	1951	% Change	1950	1951	% Change
Clinical Chemistry	4177	5686	≠ 36.1	6438	9284	≠ 44.4
Food Chemistry	179	2151	*	388	6948	*
Toxicology	621	1460	≠134.5	4449	9536	≠116.2
Water Analysis	259	311	≠ 20.7	4923	2714	- 44.9
Allergy Investigation	198	3486	*	1210	3023	*
Miscellaneous Analysis	3381	2095	**	6297	5649	**
Totals	8815	15189	≠ 72.3	23705	37154	≠ 56.7

* Not comparable. Section added last quarter of 1950.
** Not comparable due to reorganization of Department.

CLINICAL CHEMISTRY: The Clinical Chemistry Section operates as a service to Medical Corps Officers of hospitals and dispensaries within the Far East Command. This service includes not only Army medical installations, but also Air Force and Navy units. Due to the fact that most of these organizations have laboratory facilities for performing most routine examinations, the bulk of the work of the Clinical Chemistry Section has substance in the type of determination requiring either special techniques, special apparatus or other facilities which are not available to the smaller, less completely equipped unit laboratories.

The following table lists specimens received in the Clinical Chemistry Section during 1951:

Table II. Clinical Specimens Received, 1951

Specimen	No.	Specimen	No.
Blood	2060	Spinal Fluid	442
Feces	376	Urine	240
Plasma	521	Miscellaneous	87*
Serum	1960	Total	5686

* Includes calculi, gastric and pleural
fluids, thromboplastin, etc.

Table III lists the number and types of determinations completed on the specimens submitted.

During the past year, Clinical Chemistry has evaluated a number of procedures. As a result of these investigations, some procedures were discarded as being unsatisfactory and some were modified and written up as standard operating procedures for distribution to various hospital laboratories. The more important of these follow Table III.

Table III. Clinical Determinations Performed

Determination	No.	Determination	No.
Acid, Free	3	Lead, Urine	7
Acid, Total	5	Lead, Water	2
A/G Ratio	401	Lipase	38
Albumin	363	Magnesium	2
Alcohol, Ethyl	793	Methemoglobin	2
Amylase	39	Non-Protein Nitrogen	203
Bile	10	Occult Blood	374
Bilirubin	530	Pepsin	1
Bromide	7	Phenolsulphalein	7
Bromsulphalein	62	Phosphatase, Acid	56
Calcium, Blood	151	Phosphatase, Alkaline	398
Calcium, Fecal	2	Phosphorus, Blood	77
Calcium, Urinary	3	Phosphorus, Fecal	2
Calculi	29	Phosphorus, Urinary	3
Carbon Dioxide	74	Phospholipids	1
Carboxyhemoglobin	1	Porphyrins	26
Carotins	4	Potassium	140
Cephalin Flocculation	957	Prothrombin	26
Chlorides	142	Salicylates	3
Cholesterol, Total	457	Sodium	130
Cholesterol, Esters	294	Starch	1
Creatine	6	Sulfa Level	8
Creatinine	86	Sulfhemoglobin	1
Fat, Fecal, Quan.	5	Thymol Turbidity	564
Fat, Fecal, Qual.	4	Total Nitrogen	17
Fibrinogen	1	Total Protein	845
Globulin, Quan.	362	Trypsin	3
Globulin, Qual.	187	Tryptophane, Qual.	3
Glucose	216	Urea Nitrogen	136
Glucose Tolerance	36	Uric Acid	92
Hemoglobin, Blood	5	Urobilinogen	13
Hemoglobin, Plasma & Serum	509	Vitamin A	4
Icteric Index	250	Zinc, Water	1
17-ketosteroids	104	Total	9284

CHLORIDE DETERMINATIONS: The s-diphenylcarbazone indicator employed in the Schales and Schales method for chloride (1) was found to give variable endpoints with slight changes in pH. It has been recommended that the Hiller and Van Slyke modification of the Sendroy silver iodate method (2) be used routinely with the following modification:

Standardization of Thiosulfate - Into three 50 ml Erlenmeyer flasks pipette 1 ml of 0.1 N hydrochloric acid (normality of the acid must be known to four decimal places). Then carry through the entire procedure, starting with the addition of phosphotungstic acid.

$$\text{Normality of Thiosulfate} = \frac{(\text{Normality of HCl}) \ (2.308)}{\text{ml thiosulfate used}}$$

For serum, plasma, spinal fluid:

mg% NaCl = (2535) (N. thiosulfate) (ml thiosulfate used)

For 24 hour urine:

$$\text{gm NaCl} = (0.0254) \ \frac{24 \text{ hr. urine}}{(\text{volume in ml})} \ (\text{N. thiosulfate}) \ (\text{ml thiosulfate used})$$

The above modification eliminates the necessity of making a solution of an exact normality and also corrects for any chloride which may be present in the reagents.

PLASMA PROTEINS: Comparison of the Biuret procedure used in this laboratory (unpublished) and the copper sulfate specific gravity procedure for total protein against nitrogen determinations indicated that the copper sulfate procedure was the more exact. As a result of this investigation, total protein is determined by the copper sulfate procedure. Albumin is determined with the Weischselbaum Biuret Reagent (3) by comparing the unknown albumin which has been separated by sodium sulfate fractionation against a human albumin standard containing the same concentration of sodium sulfate as the unknown. Although sodium sulfate affects the Biuret color, most of the papers which have been published have evidently not investigated this effect for they have not added sodium sulfate to their standard. The Biuret procedure appears very reliable for the albumin determination. This is undoubtedly due to the fact that albumin from one individual to another is essentially the same, which is not true for the globulins and is probably the reason why the procedure is not as reliable for total protein.

DETERMINATION OF UROBILINOGEN IN URINE AND FECES: The method of Swartz, Sborov and Watson (4) for quantitative fecal and urinary urobilinogen has been adapted to routine use with only minor changes. However, the paper is extremely vague in certain respects, especially in the preparation of the standards and calculations. The procedure, as used in this laboratory, is given below:

Reagents:

1. Crystalline ferrous sulfate
2. 10% sodium hydroxide
3. Petroleum ether
4. Saturated aqueous sodium acetate
5. Ehrlich's Reagent: To a 250 ml flask add 0.7 gm paradimethylaminobenzaldehyde and 150 ml of concentrated hydrochloric acid. Shake until dissolved, then dilute to the 250 ml mark and mix.
6. Preparation of Stock Standard: Place 95 mg of pontacyl violet 6R, 5 mg of pontacyl carmine 2B, and 5 ml of glacial acetic acid in a 1000 ml volumetric flask and dilute to the mark with water. 17 ml of this solution is equivalent to 0.5 mg of urobilinogen.

7. Working Standard: Dilute 17 ml of the stock standard to 100 with 0.5% acetic acid. 10 ml of this solution is equivalent to 0.05 mg of urobilinogen.

Note: The dyes used in the standard may be obtained from Dyestuff Division, DuPont Company, Wilmington, Delaware

Preparation of Graph for Urobilinogen - In order to standardize the spectrophotometer, the following dilutions were prepared in curvettes and read at wave length 550 using water as a blank:

Cuvette No.	Ml of Dye	Ml of 0.5% Glacial Acetic Acid	Mg of Urobilinogen per 10 ml
1	2	8	.01
2	4	6	.02
3	6	4	.03
4	8	2	.04
5	10	0	.05

Density is plotted against the Concentration on graph paper.

QUANTITATIVE 24-HOUR URINARY UROBILINOGEN:

1. Collect 24-hour urine in a bottle containing about 5 gm of Na_2CO_3 under a layer of petroleum ether (20 ml).

2. In a 125 ml Erlenmeyer flask place:

   50 ml aliquot of the 24-hour urine
   25 ml of ferrous sulfate (freshly prepared 20% solution)
   25 ml of 10% naOH

   Mix well, allow to stand in the dark for one to two hours, and then filter.

3. To determine the amount of filtrate to use, do a qualitative test as follows:

   To 1 ml of filtrate, add 1 ml of Ehrlich's reagent and 3 ml of sodium acetate solution.
   If the color is intensely red, use 1-5 ml of filtrate; pale red, use 5-10 ml; faint pink, use 15-25 ml; and no color, use 50 ml of filtrate.

4. The amount selected is placed in a separatory funnel and diluted to approximately 50 ml.

   Add 5 ml glacial acetic acid and 50 ml petroleum ether. Shake vigorously for one minute.
   Separate the aqueous and ether layers. Note: If an emulsion forms, it may be broken by the addition of 2 ml of acetic acid and in some cases 2 ml of 95% alcohol is necessary.
   Extract the aqueous portion twice more with petroleum ether.
   Combine the ether solutions and wash once with 10 ml of water, discarding the water.
   Add 2 ml of Ehrlich's reagent and shake the solutions vigorously for one minute. Add 6 ml of sodium acetate and shake the solutions gently for one minute. Allow fractions to separate. Transfer the colored aqueous fraction to a 100 ml graduate.
   Repeat the extraction of the ether with Ehrlich's reagent and sodium acetate one or more times until the aqueous fraction shows only a very faint pink.
   Dilute the aqueous extracts to 25 or more ml (depending on the intensity of red color), with one part Ehrlich's reagent and three parts sodium acetate and mix. Place 10 ml in a cuvette.
   Blank: Add 6 ml of sodium acetate to 2 ml of Ehrlich's reagent in a cuvette.
   Set wave length at 550. Adjust blank to 0 on the density scale and read the unknown on the density scale.

5. Calculation:

   $$\text{mg of urobilinogen} = \frac{\text{ml(total urine vol.) ml (extract dilution) mg from gra}}{\text{ml(filtrate used) (5)}}$$

   Example: The total 24 hour urine volume was 1210. The final dilution of extract was to 30 ml. Density reading was equivalent to 0.0035 mg in 10 ml in the cuvette, and 25 ml of urine filtrate was used. Therefore:

$$\frac{(1210)\ (30)\ (.0035)}{(25)\ (5)} = 1.0 \text{ mg. per day}$$

Normal is 0-4 but is usually from 0.5 to 2 mg per day

QUANTITATIVE 24-HOUR FECAL UROBILINOGEN:

1. Collect 24 hour feces in a wide-mouthed bottle without any preservative. Bottle should be weighed previous to collection. At end of 24 hours, weigh bottle plus feces and determine the weight of the feces. Mix feces thoroughly with a spatula.

2. Weigh out 10 gm of feces and triturate with 10 ml of water. Pour the supernatant into a 100 ml graduate. Repeat the process four times. With three 10 ml portions of water, transfer the finely divided suspension of feces to the graduate and dilute to 100 ml.

3. The above suspension is poured into a 500 ml Erlenmeyer flask. Rinse out the graduate with two 50 ml portions of water, adding this to the 500 ml flask. Add 100 ml of 20% ferrous sulfate and 100 ml of 10% sodium hydroxide. Mix well, allow to stand in the dark for 1-2 hours, and then filter.

4. Place 20 ml of filtrate in a separatory funnel. Add 30 ml of water and proceed as for urine, beginning with step 4 (a through i) of the urine procedure.

5. Calculation:

   mg of urobilinogen=gm (24 hr. feces) ml (extract dilution) mg (from graph) (0.2)

   Example: The total 24 hour feces weighed 111 gms. The final dilution of extract was to 100 ml. Density reading was equivalent to 0.022 mg in 10 ml in the cuvette. Therefore: (111)(100)(.022)(.2)= 48.8 mg per day.

   Normal is 40-280 but is usually from 100-250 mg per day.

THYMOL TURBIDITY: Thymol turbidity estimation, based on the Shank and Hoagland (5) modification of MacLagan's original method (6) has been in a confused state because of a typographical error in their original paper. The barium chloride solution used in the preparation of the barium sulfate standards should be 0.0962 M (2%) instead of 0.0962 N (1%) as reported by Ducci (7).

SERUM POTASSIUM DETERMINATION: The determination of potassium has always been one of the more difficult and less accurate determinations in a clinical laboratory. Flame photometry is excellent, but the cost of such an apparatus for each hospital is still too high. With this in mind, various potassium procedures were investigated. The most commonly used procedure is that of Looney and Dyer (8). With this procedure, duplication of results on the same sample is excellent, but on normal serums the potassium values are frequently too high and recoveries run as high as 120%. This procedure used potassium sulfate as a standard and precipitates the chlorides from serum by adding an excess of silver nitrate. Thus, the standard and the unknown are not treated alike and the unknown will always have more silver ion than the standard. Aqueous solutions of potassium chloride and potassium sulfate, containing the same amount of potassium, were treated according to the Looney and Dyer procedure, and it was found that if the potassium chloride solution had even a slight amount of silver in excess of the potassium sulfate standard, it would give far higher readings.

By combining the much more simple Hoffman precipitation technique (9) which uses cobaltinitrite without the addition of silver and the colorimetric technique of Looney and Dyer (8), we obtained identical results for the potassium chloride and potassium

sulfate solutions. Recovery studies using this method were satisfactory. Although recently published papers have used the more sensitive silver cobaltinitrite reagent, this appears to be a mistake for quantitative work. The following procedure is used routinely at this laboratory and has been recommended to other laboratories in the command:

Reagents:

1. Sodium cobaltinitrite:

Dissolve 25 gm cobalt nitrate in 50 ml of water, add 12.5 ml of glacial acetic acid and stir.
Dissolve 120 gm of sodium nitrite in 180 ml of water.
To all of solution of cobalt nitrate add 210 ml of solution of sodium nitrite. Draw air through the mixture until all of the nitric oxide passes off. Keep in the refrigerator and filter each time before use. The reagent will keep at least one month.

2. Sodium acetate solution:

Saturate 250 ml of water with sodium acetate. Allow to stand over night to make sure that crystals still remain and that the solution is saturated. Filter the solution and add an equal volume of water. Mix well.

3. Ethyl Alcohol (70%): Prepare from 95% ethanol U.S.P.

4. Sulfanilamide reagent: Dissolve 0.5 gm sulfanilamide in enough 30% acetic acid to make 100 ml of solution. Prepare fresh weekly.

5. Coupler reagent: Dissolve 0.1 gm N-(1-naphthyl)-ethylene-diamine dihydrochloride in enough 30% acetic acid to make 100 ml of solution. Prepare fresh weekly.

6. Hydrochloric acid (50%): Prepare from Hydrochloric Acid, U.S.P.

7. Standard potassium solution: Prepare a stock standard by dissolving 2.229 gm. of pure dry potassium sulfate in water in a 500 ml volumetric flask, dilute with water to the mark and mix. Preserve with a little toluene. This solution contains 2 mg of potassium per ml and is stable indefinitely. Prepare a working standard fresh each week by diluting 10 ml of stock standard to 100 ml with water in a volumetric flask. This solution contains 0.1 mg of potassium in 0.5 ml.

Procedure:

1. Using two 15 ml centrifuge tubes, place 0.5 ml of serum in the first tube and 0.5 ml of standard (0.1 mg potassium) in the second. To both tubes add 0.5 ml of the half saturated sodium acetate solution and mix. Add rapidly to both tubes 1.0 ml of the freshly filtered sodium cobaltinitrite reagent and mix. Place the tubes in a water bath at 20º to 25ºC. and let stand 45 minutes to one hour but no longer.

2. Wash down the sides of the tube with 1 ml of water in such a way that the water overlays the solution. Do not mix.

3. Centrifuge at 2000 RPM for 15 minutes.

4. Invert and let drain on filter paper for 5 minutes. Wipe the mouth of the tube with a cloth while it is still inverted.

5. Wash down the sides of the tube with 0.5 ml of water, disturbing the precipitate as little as possible.

6. Centrifuge at 2000 RPM for 10 minutes. Decant, drain and wipe the mouth as before (see 4.).

7. Add 1 ml of 70% alcohol rapidly in such a way as to break up the precipitate. Wash down the sides of the tube with another 1 ml of 70% alcohol in such a way as to overlay the solution. <u>Do not mix</u>.

8. Centrifuge, decant, etc. (see 6.).

9. Add 2 ml of 0.2 N sodium hydroxide rapidly in order to break up the precipitate. Then add 8 ml of 0.2 N NaOH. Place in a boiling water bath for 10 minutes, cool and make up to the 10 ml mark with water.

10: Mix the solution by inverting, stopper to prevent evaporation, and centrifuge as in 3 above.

11. Development of color: Use three 100 ml volumetric flasks as follows:

#1 Unknown	#2 Standard	#3 Blank
5.0 ml water	5.0 ml water	5.5 ml water
0.5 ml supernatant from ctfg tube	0.5 ml supernatant from ctfg tube	

To all flasks add 2 ml of sulfanilamide solution and 1 ml of 50% hydrochloric acid. Mix by swirling (do not invert) and let stand for 3 minutes. Then add 1 ml of the coupler reagent and dilute to the mark with water.

12. Let stand 10 minutes and read the density at wavelength 540.

Calculation: $\dfrac{U}{S} (20) = $ mg of K per 100 ml serum

The graph shown in Figure 1 was prepared by carrying through the entire procedure using potassium concentrations equivalent to 10, 20 and 30 milligrams percent.

If the temperature of precipitation and color development was controlled, a constant factor could probably be used instead of carrying through a standard with each series of unknowns. However, standards are preferred by this laboratory rather than using constant factors, since the use of standards gives better control of the procedure.

PLASMA HEMOGLOBIN: In January, the Clinical Chemistry Section was called upon to determine the hemoglobin content of a large number of plasma samples. Investigation of the Bing and Baker method (10) showed that it was applicable to the problem. However, it was necessary to make some modifications to adapt the procedure to the spectrophotometer.

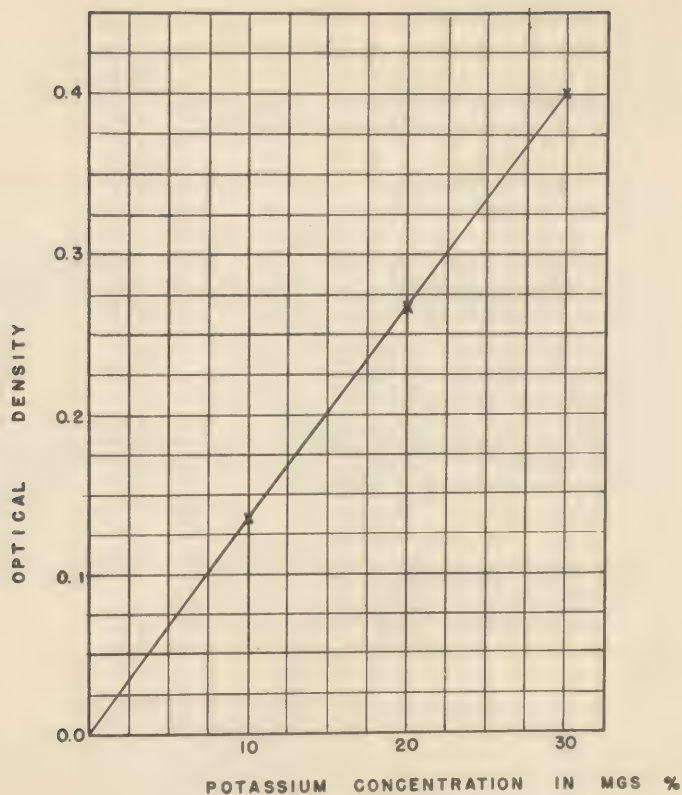The modified method as used at this laboratory follows:

Figure 1. Standard Curve for Potassium

Hemoglobin Standard - Accurately determine the hemoglobin concentration of a sample of whole blood, preferably using one of the gasometric methods. Using distilled water, prepare dilutions containing 50, 75 and 100 gammas of hemoglobin per ml.

Reagents -

1. 2% benzidine in glacial acetic acid.

2. 20% acetic acid.

3. 0.6% aqueous hydrogen peroxide solution. This solution must be accurately prepared, preferably from a 3% solution that has been assayed by the method in U.S.P. XIII, page 260.

Procedure - Into spectrophotometer tubes pipette (in the order given):

1. 0.6 ml of benzidine reagent.

2. 0.2 ml of standard or unknown.

3. 0.2 ml of hydrogen peroxide reagent.

Allow to stand for one hour. Add 5 ml of 20% acetic acid solution, mix and allow to stand for an additional 15 minutes. Read in the spectrophotometer at 640 mu using the 10 gamma standard as the blank.

On graph paper 10 gamma, 15 gamma and the 20 20 gamma standards are plotted against their respective densities. This graph is used to determine the concentration of the unknowns.

With every run of unknowns, the three standards are run. If the color developed by the unknown does not fall between that of the 10 gamma and the 20 gamma standards, a new dilution of the unknown is made and the procedure repeated.

When the density is plotted against the concentration, a straight line is obtained between 10 and 20 gamma. However, this line does not go through the origin and above 20 gamma the line flattens out. The wavelength used (640) is one of minimum absorption since the color developed is too strong to be read at the wavelength of maximum absorption. Attempts to use more dilute solutions, read at a wavelength of maximum absorption, failed because extremely low concentrations of hemoglobin produced erratic reactions.

Standard operating procedures for the above and the following determinations have been sent to many Army and Air Force hospitals in this command: Quantitative direct (one minute) and total bilirubin, blood hemoglobin, urea nitrogen of blood and urine, total urine nitrogen using selenium as a catalyst, serum sodium, and others. In addition, this laboratory furnishes evaluation standards upon request.

TOXICOLOGY: The year 1951 brought a considerable increase in the workload of the Toxicology Section. A total of 1460 specimens were examined on 201 autopsies and 419 clinical cases. This is a 135% increase over the number of specimens received in 1950. The number of determinations performed increased from 4449 to 9536 this year, for an increase of 116%. The rate of increase has been progressive with the first two months producing 65 requests and the last two months producing 152.

During the year new methods have been adopted and additional equipment secured, making it possible to improve the toxicological examinations performed.

Table IV indicates the number and types of specimens received, while Table V shows the various examinations that have been required on the specimens received.

Table IV. Toxicology Specimens Received

Specimen	No.	Specimen	No.
Blood	390	Paper	1
Brain	79	Pericardial Fluid	1
Cigarettes	2	Plant Material	2
Crystalline Material	18	Pleural Fluid	1
Drugs	11	Small Intestine	42
Fluids, Alcoholic	23	Spinal Fluid	1
Foods	3	Spleen	3
Gastric Lavage	10	Spoons	3
Heart	3	Stomach Content	150
Hypodermic Syringes	10	Tissue, mixed	53
Intestinal Content	17	Tobacco	1
Kidney	91	Unknown Fluids	11
Large Intestine	2	Urine	407
Liver	105	Vomitus	15
Lung	3	Water	2

Table V. Toxicology Examinations Completed

Determination	No.	Determination	No.
Acetaldehyde	8	Formic Acid	8
Acetone	3	Glass	80
Alcohol, Ethyl	625	Halogenated Compounds .	422
Alcohol, Methyl	568	Heavy Metals	323
Aldehydes	687	Heroin	39
Alkaloids	2120	Isopropyl Alcohol	3
Arsenic	43	Magnesium	6
Atropine	2	Marihuana	10
Barbiturates (qual.)	1061	Meconic Acid	1
Barbiturates (quan.)	36	Mercury	1
Benzedrine	2	Morphine 2174	
Benzene	2	Nicotine	2
Bromides	7	Nitrates	4
Cantharides	4	Novocaine	2
Carbon Monoxide	21	Non Protein Nitrogen ..	2
Carbon Tetrachloride	18	Opium	2
Chloral Hydrate	11	Oxalates	5
Chlorides	12	Papaverine	6
Chloroform	2	Phenobarbital	2
Codeine	4	Phenols	656
Cocaine	10	Salicylates	2
Cyanide	421	Saponins	4
Dramamine	2	Silica	2
Ephedrine	2	Sodium Bicarbonate	4
Ethylene Glycol	7	Strychnine	9
Formaldehyde	85	Vanillin	4
		Total	9536

A brief review of the significant positive findings is given below.

Ethyl Alcohol - Significant ethyl alcohol concentrations were found in 29% (58 out of 201 cases) of the autopsies examined during the year. This is a decrease from the 40% reported in 1950.

Carbon Monoxide - Six autopsy cases gave high carbon monoxide results. The case numbers and percent hemoglobin saturation are given below:

         Case 179 - 75% hemoglobin saturation
         Case 1631 - 68% hemoglobin saturation
         Case 2040 - 31% hemoglobin saturation
         Case 3884 - 83% hemoglobin saturation
         Case 6993 - 90% hemoglobin saturation
         Case 7143 - 40% hemoglobin saturation

Heavy Metals - Arsenic was detected in four instances. One case showed a concentration of 118 gamma of arsenic per 100 gm of liver tissue. The urine in one case contained 60 gamma of arsenic per 100 ml. Arsenic was detected in two samples of vomitus submitted for examination.

Chlorides - Comparison of chlorides in blood from left and right ventricles was made in six cases of suspected drowning.

Cyanide - One case of cyanide poisoning was encountered during the year. Quantitative determination gave the following results:

```
Stomach Content ................... 400 gamma per ml
Blood ........................... 100 gamma per ml
Brain ...........................   2 gamma per gm
Liver ...........................   1 gamma per gm
```

Carbon Tetrachloride - This substance was found in the stomach content of a
child who had consumed an unknown fluid.

Formaldehyde - Heat tablets, consisting of either 1,3,5 trioxane or hexameth-
ylenetetramine, both polymers of formaldehyde, were responsible for deaths in two cases.
Formaldehyde was demonstrated in the fresh tissue examined.

Methyl Alcohol - This substance was detected in the tissues and fluids sub-
mitted on 13 cases during the year. Tissue levels from 0.1 mg/gm to 2.0 mg/gm were en-
countered. Submitted with the tissues in one case was a sample of fluid which had
caused death of six of the ten men who drank from the bottle. The fluid was identified
as methyl alcohol.

Glass - Forty sections of small intestine were examined for the presence of
ground glass. After grinding in a food chopper, the sections were subjected to Kjel-
dahl digestion and then left to stand overnight. Settled residue from the bottom of
the flask was transferred to a graduated centrifuge tube and centrifuged for 10 min-
utes. The supernatant was carefully removed with a pipette and the residue was washed
several times with 10 ml portions of water by shaking, centrifugation, and removal of
the supernatant. The final residue was transferred to a glass slide and examined mi-
croscopically. Visible, refractile particles were subjected to fusion on platinum
foil to distinguish between glass and other forms of silica. Twenty of the sections
yielded positive results. The method used is essentially that of McNally (11) with
one exception. The Kjeldahl method of digestion was found more satisfactory than the
Fresenius and Von Babo method recommended by McNally. Great care was exercised in the
use of glassware with inspection before and after use to insure that glass recovered
had not originated from equipment used. Numerous normal sections of intestine were
used as controls and subjected to the same treatment. Negative results were obtained
on control specimens.

Dramamine - In one case of attempted suicide, dimenhydrinate (dramamine) was
isolated from the stomach washings and identified as the drug used. Identification
tests are based on the fact that dimenhydrinate is closely related to diphenylhydram-
ine, an antihistaminic agent. The drug was extracted from stomach washings with alco-
hol. After evaporation of the alcohol, the residue was taken up in 15 ml of water
and 2 ml of strong ammonia solution was added. The free base was extracted from the
resulting solution with several 10 ml portions of ether. After washing the combined
ether extracts with 5 ml of water, 1 ml of concentrated HCl was added to the ether
solution. The liquids were mixed and warmed on the steam bath to expel the ether and
most of the water. The residue responded to the identity tests for diphenylhydramine
hydrochloride as given in U.S.P. XIV.

Diethylene Glycol - This substance was shown to be the cause of death in two
cases in which a fluid believed to be anti-freeze solution was consumed. Method of
detection was based on the reaction for polyhydroxy alcohols as given in Feigl (12).
The polyhydroxy alcohols are converted to formaldehyde and formic acid by treatment
with excess periodic acid in the cold. After the destruction of excess periodate and
iodate with sulfurous acid, the formaldehyde is detected with fuchsin-sulfurous acid.
Aldehydes and some carbohydrates, which also give this reaction, must first be ruled
out. Tartaric acid gives the reaction and should not be used in a steam distillation
designed to eliminate aldehydes and other volatile materials.

In a sample of stomach content, filtered to remove insoluble materials, most of
the carbohydrates and aldehydes present can be removed by steam distillation. Using
a strong mineral acid the di- and polysaccharides are hydrolyzed to form monosacchar-
ides which in turn are converted by loss of water to aldehydes which are steam

volatile. Residue from distillation contains the relatively non-volatile ethylene glycol which can be identified by the method given in Feigl (12).

Miscellaneous Specimens - Numerous specimens, including crystalline materials, syringes, needles, mess kit spoons, tablets, and cigarettes were examined for evidence of narcotics during the year. Among the agents found in or on the specimens were heroin, morphine, opium, codeine, narcotine, and several of the barbiturates.

Alkaloids and Barbiturates - Twenty-five percent (50 out of 201) of the autopsy cases examined yielded positive results for alkaloids or barbiturates. Alkaloids were found in 21 cases and barbiturates in 29. Morphine was the alkaloid most commonly recovered. Nicotine was found responsible for death in one case and lobeline, codeine, and apomorphine were identified in other cases.

Fluids were submitted from 404 individuals suspected of using narcotics. Alkaloids were detected in 102 cases and barbiturates in 85 cases, for a total of 187 positive cases or 46% of the examinations. Again, the alkaloid most commonly identified was morphine. Many cases were received in which the size specimen submitted was hardly sufficient to permit proper examination but insofar as possible they were examined and the originating agency informed of the importance of submitting larger specimens. It was necessary to return to origin 15 unexamined requests in which as little as 1.5 ml of blood and 10 to 15 ml of urine were submitted. Experience at this laboratory has indicated that recoveries of narcotics can best be effected if a minimum of 20 ml of blood and the entire first voiding of urine following apprehension of the suspect are furnished on clinical cases. For autopsy investigations at least 200 gm each of kidney, liver, and brain as well as total stomach contents, urinary bladder contents, and 40-100 ml of blood have been found to be adequate for most examinations.

A considerable amount of effort was devoted to work on alkaloids and barbiturates during the past year. The ready availability of drugs in this area necessitates examination for alkaloids and barbiturates in most specimens received for toxicological studies. The Stas-Otto procedure (13) for the extraction of alkaloids from body tissue is used with minor variations. When available, a specimen of fresh tissue is minced and extracted rather than using residue from steam distillation as recommended by most authors. Good cooperation of the submitting agencies has made this possible in most cases. Large specimens have been requested and received on autopsies which are accompanied by a protocol containing available information regarding the circumstances of death. Occasionally, it has become necessary to resort to the more complicated method of extraction involving refluxing with alcohol as outlined by McNally (14). Since this method requires more individual attention, it is only used in autopsy cases where evidence points to alkaloids as the responsible agent. Although the method has proved more sensitive, the attention required renders it impractical where large numbers of specimens are processed. An alkaloid is never reported as present unless the usual color reactions can be substantiated by specific crystalline reactions with precipitating reagents. At times it has been necessary to further purify extracts to demonstrate satisfactory color reactions. Most color reactions as reported in literature are excellent with pure material but fail miserably in the presence of impurities. In this respect the literature reports are misleading since they deal only with pure compounds. Many of the color reactions described in the literature are also given by putrefaction products of tissues. Tissues are often received in an advanced state of putrefaction and alkaloid identifications can be reliable only by the combination of color reactions in conjunction with the more specific crystalline reactions. Sublimation techniques have been employed successfully in obtaining relatively pure crystalline forms of alkaloids from tissues. Periodic checks are made to insure that all procedures in use are the most accurate and sensitive available. Known amounts of volatile and non-volatile poisons are occasionally added to normal specimens which are then submitted to our standard procedure for the isolation and identification of poisons. This offers an excellent opportunity for the evaluation of procedures and technicians.

Halogenated Compounds - One method examined and placed in use this year is Ross's alkaline pyridine test for halogenated compounds (15). Using reagent grade pyridine and 40% potassium hydroxide, the sensitivity is reported by Merley and Bueding (16) to be 0.0002 percent for chloroform, 0.0001 percent for chloral hydrate, and 0.025 percent for carbon tetrachloride. The results are more easily interpreted than those given by Schwartz's test using resorcinol and 10% sodium hydroxide as given in Simmons and Gentzkow (17).

FOOD CHEMISTRY: The Food Chemistry Section, which was established late in the year of 1950, functions as a quality control laboratory in the analysis of recombined milk products produced by the several processing plants in Japan, in the analysis of components of the South Korean field ration, and other food products from local Japanese contractors. It functions as a nutritional laboratory in the analysis of foods for proteins, fats, carbohydrates, fiber, ash, moisture, calorie content and ascorbic acid.

Space is a limiting factor in the amount of work that can be accomplished. Methods of analysis follow the procedures of "Standard Methods of Analysis of the Association of Official Agricultural Chemists" (AOAC), 6th Edition, 1945, where possible. It has often been found necessary to adopt methods with modification from other standard tests due to the nature of the sample received, for which no method is to be found in AOAC. Samples analyzed and types of determinations accomplished are shown in Tables VI and VII.

Table VI. Food Chemistry Specimens

Sample	No.	Sample	No.
Ammonium Carbonate	6	Flour:	
Banana Oil Flavoring	1	Blended, Enriched	1
Beef and Beans, Canned	1	Hard Wheat	16
Beef, Corned, Canned	1	Rice	2
Beef, Ground	6	Rye	1
Beef Hash, Canned	1	Soft Wheat	1
Beef Stew, Canned	1	Grape Juice, Canned	1
Biscuits:		Horse Blood, Dried	2
Field, ROKA	207	Kelp, Dried	57
Field, TOG	1	Mackerel in Olive Oil, Cnd ...	1
Turkish Ration, Cnd	6	Mackerel Pike, Canned	7
Biscuit & Candy, Cnd. ..	2	Meat with Beans, Canned	6
Butter, Canned	1	Oleomargarine	2
Chinese Combat Ration:		Olive Oil	1
Rice Biscuit	1	Orange Peel	2
Dried Beans	1	Peppers, Red:	
Sugar Product (Candy) ..	1	Canned	1
Clams, Fresh, Frozen	4	Dried	1
Codfish:		Dried, Ground	2
Bodara	3	Hontaka, Whole	1
Dried	38	Santaka, Whole	1
Canned	7	Pickles, Dill, Canned	1
Madera	2	Pickles, Sweet, Canned	2
Sukesudara	7	POW Ration-3 meals each	7
Cuttlefish, Dried	24	Prune Juice, Canned	1
Daikon, Dried	1	Ration, Hospital-3 meals	2
Dairy Products:		Rennet	6
Chocolate Milk	280	Rice Starch	31
Cream, Half and Half ...	55	Salt	1
Egg Nog	2	Sardines, Canned	3
Ice Cream	581	Soya Oil	3
Ice Cream Mix, Pwd	33	Sugar	14
White Milk	659	Tuna Flakes, Canned	7
Flour:		Yeast, Dry, Active	6
Biscuit Mix	1	Yolk Cheese	26
		Total	2151

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Table VII. Food Chemistry Determinations

Determination	No.	Determination	No.
Acidity as Acetic Acid	2	Iron, Total	3
Acidity as Olive Oil	8	Lactic Acid	2
Acid Value of Olive Oil	8	Mathew's Dye Test	1
Alcohol	3	Moisture	754
Arsenic	2	Organaleptic	6
Ascorbic Acid, Total	11	pH	19
Ash	103	Phosphorus	2
Assay, Ammonium Carbonate	6	Protein	106
Brix	1	Refractive Index	1
Calories	2	Reinsch Test	2
Carbohydrate	88	Rennin, Quan.	2
Carbon Dioxide	3	Saponification Value	6
Copper	4	Sediment	725
Drained Weight	2	Size and Fill of Can	1
Fat	1761	Sodium Chloride	10
Fermentation CO_2 Yeast	1	Sodium Nitrite	4
Fiber	80	Solids not Fat	1592
Gas Measurement Swollen Cn	4	Specific Gravity	3
Glycogen	1	Titratable Acidity	4
Halphen Test	2	Total Acid	11
Hexabromide Number	1	Total Solids	1592
Iodine Value Hanus	6	Unsaponifiable Matter FAC .	6
Iron, Available	2	Wool Dye Test	1
		Total	6948

Samples have been received at a fairly constant rate of about 175 per month throughout the year. There has been a constant increase in the number of dairy products received for analysis and a decrease in other products during the last quarter.

The analysis of dairy products in the quality control of recombined milk, chocolate milk, ice cream and cream comprised a large part of the work. A summary of the analyses in comparison with last year appears in Table VIII.

Table VIII. Examination of Dairy Products

Product	Year	No. of Samples	No. of Samples Low in Butter Fat or Total Solids	Percentage Low
White Milk	1950	53	8	15.1
White Milk	1951	659	106	16.1
Chocolate Milk	1950	42	5	11.9
Chocolate Milk	1951	280	14	5.0
Ice Cream	1950	32	3	9.3
Ice Cream	1951	580	95	16.4
Cream	1950	None		
Cream	1951	55	15	27.3
All Dairy Products	1950	137	16	11.7
All Dairy Products	1951	1574	230	14.6

Dairy Products - The results of analyses of duplicate line samples sent to the contractor and analyzed in this laboratory are often not in agreement, especially in those samples in which there is a low butter fat content. The methods used by this laboratory are the official methods of AOAC, specified in the contract, while those of the contractor are the Dietert Method employing forced heated air at 275°F. The Dietert Method is not recognized as an official method in AOAC, but rather is a rapid method with but relative accuracy, whereas the official method, although much slower, has been proved accurate and reproducible for many years. It is difficult for the contractor to understand why he cannot produce the same results with his rapid method that are produced with the official method. The difference has been explained several times and duplicate samples exchanged for his benefit.

Prisoner of War Rations - One P.O.W. ration composed of the three meals served in one day, was received in February and six similar rations were received in June for analysis. The rations were obtained from the cafeteria style mess lines and were said to represent average servings. The improvement of the June rations over the February rations, both in total calories and the amount of fat and protein contained in the June rations, indicates that there has been a change in the basic components of the ration. The composition of the rations was as shown in Table IX.

Table IX. Prisoner of War Ration Analysis

Ration	Protein*		Fat		Carbohydrates**		Calories***
	Grams	Calories	Grams	Calories	Grams	Calories	
February Ration	44.59	182.8	2.95	27.4	375.97	1541.5	1751.7
June Ration No. 1	82.10	336.6	62.49	581.2	430.0	1763.0	2680.8
2	92.19	378.0	15.65	145.5	471.1	1931.5	2455.0
3	93.33	382.7	13.70	127.4	565.4	2318.1	2828.2
4	68.21	279.7	11.10	103.2	492.4	2018.8	2401.7
5	156.35	641.0	75.97	706.5	415.3	1702.7	3050.2
6	81.96	336.0	33.00	306.9	469.1	1923.3	2566.2

* Nitrogen x 6.25 = Protein
** Carbohydrate obtained by difference
*** The factors of 4.1 for protein and 9.3 for carbohydrate.
were used to convert grams to calories

Methods of Special Analysis - There are no standard methods for determining the moisture content of many products received for such analyses. Products such as dried kelp, yolk cheese and dried fish were being purchased with a maximum moisture content as part of the contract, but the method by which the moisture was to be determined was not specified. The use of a vacuum oven might have been the answer, but this equipment was neither available nor was space available for one. The air oven method of drying was tried but soon found to be very inaccurate at temperatures ranging from 90o to 135°C. Duplicate results could not be obtained in the oven and the sides of the drying dishes were stained, indicating a loss of components other than moisture due to the heat of the oven. The vacuum method (18) using freshly boiled sulfuric acid in a desiccator, was investigated. The method leaves much to be desired in that it requires several desiccators and many evaporating dishes, as well as considerable time to obtain results. The drying period in the case of kelp is seven days or longer. The results with this method are lower than those obtained with the air oven, but probably closer to the true moisture content of the product. Yolk cheese and dried cod or cuttlefish can be dried in 64 hours with this method.

In order to shorten the time of the determination, the Toluene Distillation Method (19) was investigated. It was tried with kelp, which is a most difficult product to handle as it cannot be chopped or ground to a suitable degree of fineness. We have found that the best method is to cut the kelp into small pieces about 1/8 inch square with a sharp knife. One or two gram samples of these relatively large sized pieces are not too representative of the whole sample, and larger samples require an excessive drying time;

10 gram samples lose moisture for as long as 14 days. The Toluene Method yielded slightly higher results than did the vacuum method. Results of moisture determinations by the two methods are shown in Tables X and XI. Although the moisture content in most cases is higher with the Toluene Method, in Table X, samples No. 7 and 12 showed a lower moisture content. Since the samples were in a rather coarse state, it was decided to increase the time from one hour to one and one-half hours with the Toluene Method. The increase in moisture was considerable in each case, as shown in Table XI. Further increases in the distillation time did not increase the moisture content.

Table X. Moisture in Kelp

Sample No.	Vacuum Method* Percent Moisture	Toluene Method** Percent Moisture
1	12.94	14.22
2	13.65	15.98
3	13.55	15.66
4	14.58	16.19
5	11.63	14.56
6	14.32	16.27
7	16.98	16.58
8	17.42	17.48
9	16.49	17.57
10	17.04	17.35
11	16.32	19.39
12	17.25	16.60

* Dried in vacuum, 14 days
** Distilled one hour

Table XI. Moisture in Kelp

	Percentage Moisture		
	Determination		
Sample No. 1	1st	2nd	Average
Vacuum Method:			
192 hr.	22.31	22.91	22.61
240 hr.	23.20	22.52	22.86
336 hr.	23.58	22.90	23.26
Toluene Method			
1 hr.	22.90	23.30	23.10
1½ hr.	25.23	25.55	25.39
Sample No. 2			
Vacuum Method:			
192 hr.	21.90	22.21	21.56
240 hr.	22.12	22.52	21.82
336 hr.	22.48	21.88	22.18
Toluene Method:			
1 hr.	23.30	24.75	24.03
1½ hr.	25.11	25.09	25.10
Sample No. 3			
Vacuum Method			
192 hr.	33.67	33.44	33.56
240 hr.	33.79	33.51	33.65
336 hr.	34.00	33.70	33.85
Toluene Method:			
1 hr.	30.78	31.62	31.20
1½ hr.	34.20	34.20	34.20

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The advantages of the Toluene Method are: Larger, more representative samples of about 10 grams, as compared with the smaller 2 gram samples, may be used; the time required is reduced from 7 days to 1½ hours; only one weighing of the product is necessary, whereas at least two weighings are required with the vacuum method. The disadvantages of the method are: The receiver tube is scaled in 0.1 cc. necessitating an estimate to less than that figure; the condenser and receiving tube must be absolutely chemically clean or droplets or water will adhere to the sides of the tube. These droplets are removed with difficulty, if at all. To overcome this the condenser and tube are best cleaned with soap and water, followed by rinsing in tap water and then with a cleaning solution composed of one part nitric acid and three parts sulfuric acid. The cleaning solution is used hot - about 80°C. The apparatus is then rinsed in distilled water and dried.

Satisfactory extraction equipment was not available for lactic acid extraction by the ether method as described in AOAC (20) for the lactic acid determination in butter.

Barker and Summerson's method using p-hydroxydiphenyl as described by Winton (21) for use with meat was modified for butter by first removing the butterfat with ether and petroleum benzine as in the official method. The proteins were then removed with trichloracetic acid. The filtrate was handled as in the original method.

Samples of red peppers, canned, whole, and dried, were submitted for ascorbic acid determinations, as were samples of fresh and partially dried orange peel. The three methods investigated were: Martini and Bonsignore's methylene blue volumetric method as described by Winton (22); Falkmann's indophenol colorimetric method as described by Winton (23); and Roe and Kuether's phenylhydrazine method as described by Hawk, Oser, and Summerson (24). The methylene blue method failed to give satisfactory results. We were never able to reduce the methylene blue-ascorbic acid solutions by sunlight as described. Roe and Kuether's phenylhydrazine method proved less accurate and the results not as reproducible as was Falkmann's indophenol method. The results of the latter method were quite reproducible and recoveries using ascorbic acid added to the macerated peppers were excellent. In the original method the product is ground with metaphosphoric acid and water, filtered, and made up to volume. The filtration process is slow and incomplete. This was corrected by centrifuging and decanting the supernatant fluid. Several grindings and washings are necessary for a thorough extraction in the case of the peppers. With orange peel the Waring Blender is used in lieu of grinding in the mortar. Both products contain a large amount of highly colored oil which is removed before the sample is made up to volume with xylene. After extracting the oils, the sample is made to volume, the dye is added, and its color extracted with xylene and compared with standards using known amounts of ascorbic acid and the same amount of dye in the Coleman Jr. Spectrophotometer at 540 mu.

No ascorbic acid can be demonstrated with indophenol in the primary xylene extraction. The l-ascorbic acid was determined on an aliquot of the original solution. The dehydroascorbic acid was determined on another aliquot after it was reduced with hydrogen sulfide, the excess sulfide being removed with a stream of carbon dioxide. Results of the analyses are contained in Table XII

Table XII. Vitamin C Determinations

Sample No.	Product	mg/100 gm l-ascorbic acid	mg/100 gm dehydroascorbic acid	mg/100 gm total ascorbic acid
5125	Red Peppers, Canned	79.4	25.9	105.3
5244	Red Peppers, Dried	9.2	9.2	18.4
5404	Red Peppers, Dried	10.0	7.6	17.6
6261-1	Red Peppers, Dried, Ground	6.2	12.4	18.6
6261-2	Red Peppers, Fresh	20.6	2.8	23.4
6261-3	Red Peppers, Fresh	17.5	2.3	19.8
6921-1	Orange Peel, Fresh	41.6	67.4	109.0
6921-2	Orange Pell, Dried	147.0	98.0	245.0

One sample of dried red peppers, No. 5404, of Korean origin, was received with a request for ascorbic acid analysis and tests for adulteration. The sample was submitted wrapped in a piece of white bond writing paper which was stained with oil from the peppers as well as several small areas that were stained a light pink. The ascorbic acid content was within normal limits. No foreign material was found on microscopic examination. A water soluble pink dye had been added. The dye was partially identified by Mathewson's test for dyes as a disazo dye. Wool dyeing tests indicated it was not one of the permitted dyes of the U. S. Food and Drug Administration. The practice of dyeing food in Japan is not unusual and is practiced as a custom or to increase the "eye-appeal" of the product rather than to deceive the customer. Some of the products that are dyed are sliced daikon, Chinese radishes, octopus (dyed in the cooking process), fresh eggs, and ginger. All are dyed various shades of red. Three samples of dyes usually used in the dyeing process have been obtained for study and identification for future reference.

One sample of chopped meat, No. 6847, was submitted for the detection of horse meat in the sample. Precipitins for the test were not available, nor did time or space permit their preparation. Hynds' method (25) is based on demonstrating glycogen in horse meat and not in other animals and also on the high linolenic acid content of horse fat as compared with the meat of other common animals. Authentic samples of chopped horse meat and beef were procured and used as controls. The sample was negative for horse meat, as shown in Table XIII.

Table XIII. Examination for Horse Meat

Sample	Test for Glycogen	Hexabromide Number
Authentic Beef	Negative	1.2
Authentic Horse Meat	Four Plus	109.0
Sample 6847	Negative	1.1

Miscellaneous analysis of food included the analysis of the Turkish and Korean (ROKA) combat rations and two hospital rations for moisture, protein, fat, carbohydrates, ash and fiber; potency test on dried yeast and rennet tablets; the copper and iron in ice cream mix powder; the determination of a gas (carbon dioxide) in steak sauce; the assay of ammonium carbonate; the Hanus iodine value (26), saponification and unsaponifiable matter in olive oil; the iron, copper, phosphorus and protein in dried horse blood.

WATER ANALYSIS: The Water Analysis Section received 186 specimens during the year. Of these, 181 were examined for requirements as potable water, and 5 samples were from boiler water sources. Methods of analysis used were those standard methods approved by the American Public Health Association (27). In addition to the previously described specimens, 125 samples of water were analyzed for a special project which was designed to test the efficacy of various types of deionizing units for production of water of low dissolved solids content.

Table XIV lists the determinations completed by Water Analysis Section during 1951.

Water Deionization Systems - During the month of February, a special problem of evaluating an ion exchange unit, designed for producing water of low dissolved solids content, was undertaken. This unit, known by the trade name of "LaMotte Filtr-Ion Model W" is produced by the LaMotte Chemical Products Company, Towson, Baltimore, Maryland. The unit was furnished with a sufficient amount of extra resin to allow one complete refill of the exchange chamber. Apparatus, as furnished, consisted of a transparent, flexible plastic tube filled with a blue-colored ion exchange resin. At either end of the resin column there was a 1-2 mm layer of glass wool to prevent the escape of resin. Both ends were closed with a flexible rubberlike plastic cap. The top cap contained an inverted flanged hole of approximately 1.5 cm. diameter which served for connection to a water faucet. The top cap also contained three small holes of 3 mm

Table XIV. Water Analysis Determinations

Determination	No.	Determination	No.
Alkalinity, Caustic	6	Manganese	42
Alkalinity, Methyl Orange ..	114	Nitrogen, Ammonia	103
Alkalinity, Phenolphthalein.	53	Nitrates	41
Aluminum Oxide	2	Nitrites	41
Ammonia	61	Oxidizing Substances	121
Calcium	175	Odor	16
Carbon Dioxide	121	pH	305
Chlorides	175	Reaction, Bromothymol Blue	121
Chlorine Demand	12	Residue, Dissolved	51
Chlorine, Residual	3	Residue, Fixed	51
Color	41	Residue, Suspended	51
Hardness (Fe, Ca, Mg)	6	Residue, Total	363
Hardness, Non-Carbonate	4	Silica	174
Heavy Metals	121	Sodium and Potassium	5
Iron Oxide	2	Sulfates	173
Iron and Aluminum	52	Taste	9
Magnesium	54	Turbidity	45

Total   2714

diameter, designed to regulate water flow. The bottom cap had an attached flexible nozzle and tube of plastic, approximately 15 inches in length and 3 mm in diameter, which was designed to furnish a constant rate of flow through the exchange unit. Advertising information accompanying the unit gave purchase price as three dollars and eighty-five cents for the complete unit. No price for refill resin was included, but it was indicated as available.

Sixty-five liters of water were collected using the LaMotte Filtr-Ion unit and these were checked for total solids and pH, according to U.S.P. XIII (28). The rate of flow through the unit was adjusted to one liter for 35 to 40 minutes. Results of chemical examination are shown in Table XV.

Results of the examination examination indicated that the apparatus produces a deionized water suitable for the requirements of pharmacies and laboratories if the procedure as outlined by the manufacturer is followed. The unit was found to produce the results claimed by the manufacturer. One advantage of this unit is that the blue-colored dye changes to tan color when it has lost its exchange ability and, from the tests done, the water produced appears to be satisfactory until the blue color has completely disappeared. Such deionized water is not necessarily pyrogen-free.

The price of three dollars and eighty-five cents as quoted for the unit is reasonable since the unit is capable of producing approximately 50 liters of water from source water of 125-150 ppm total solids. This cost is approximately eight cents per liter of distilled water, which is less than the cost of gasoline to produce equivalent distilled water by distillation with the field distillation unit. The apparatus lends itself to easy improvisation and may be used in the absence of tap water, if necessary, in that any container suspended to allow gravity flow could be used. For those units requiring small amounts of distilled water, this appears to be an economical means of producing water of low solids content. Water produced by this unit met the requirements as outlined by U.S.P. XIII, (28).

Work on double-bed and mono-bed deionizers, designed by this laboratory and manufactured by courtesy of Japan Organo Company, Ltd., Tokyo, was begun. Preliminary investigation has produced promising results.

The double-bed deionizer is composed of separate columns of anion and cation exchange resins (Fig. 2). Source water is passed through a column of anion exchange

resin which, in theory, adsorbs the negatively charged ions from solution and re-places them with a hydroxide ion. This effluent is subsequently passed through a column of cation resin to remove the positively charged ions such as calcium, mag-nesium, iron, aluminum, etc. which are replaced by hydrogen ions from the cation resin. Hydrogen ions are neutralized by the hydroxide eluted from the anion column to form highly un-ionized water.

Table XV. Examination of Deionized Water

Sample	Total Solids (ppm)	Sample	Total Solids (ppm)	Sample	Total Solids (ppm)
1	3	23	14	45	8
2	1	24	12	46	25
3	7	25	14	47	23
4	6	26	7	48***	59
5	8	27	8	49	71
6	9	28	9	50	36
7	3	29	5	51	50
8	6	30**	9	52	50
9	8	31**	9	53	52
10	10	32	7	54	39
11	15	33	8	55	49
12*	37	34	10	56	51
13*	27	35	11	57	58
14	15	36	15	58	54
15	10	37	17	59	59
16	13	38	9	60	48
17	11	39	11	61	52
18	18	40	9	62	43
19	15	41	20	63	61
20	14	42	13	64	55
21	13	43	22	65	58
22	13	44	28	Source Water	125

All samples of water collected were within the limits spec-ified by USP XIII for pH.

* Collections of second day, without allowing the apparatus to be flushed.
** Collections made third day, allowing the first half liter to be discarded after flushing the apparatus.
*** Collections subsequent to sample 48 were made after the resin was completely devoid of blue color.

Regeneration of the multiple-bed apparatus is easily accomplished by flushing the anion exchange column with a suitable alkali. Sodium hydroxide and sodium bi-carbonate have been used here, depending on the type of anion exchange resin em-ployed. Strong base exchange resins require a strong alkali such as sodium hydrox-ide for regeneration and the weak base exchange resins may be activated by bicar-bonates.

By similar procedure, the cation resin may be recharged by using an acid. Sulfuric or hydrochloric acids have been found satisfactory for strong acid ex-change resins. After flushing with the appropriate regenerant, the columns are rinsed with tap water and the system is ready for re-use. When each column con-tains approximately 50 grams of resin, a 5% solution of acid or base may be used for each column respectively. The flow is regulated to allow one liter of the 5%
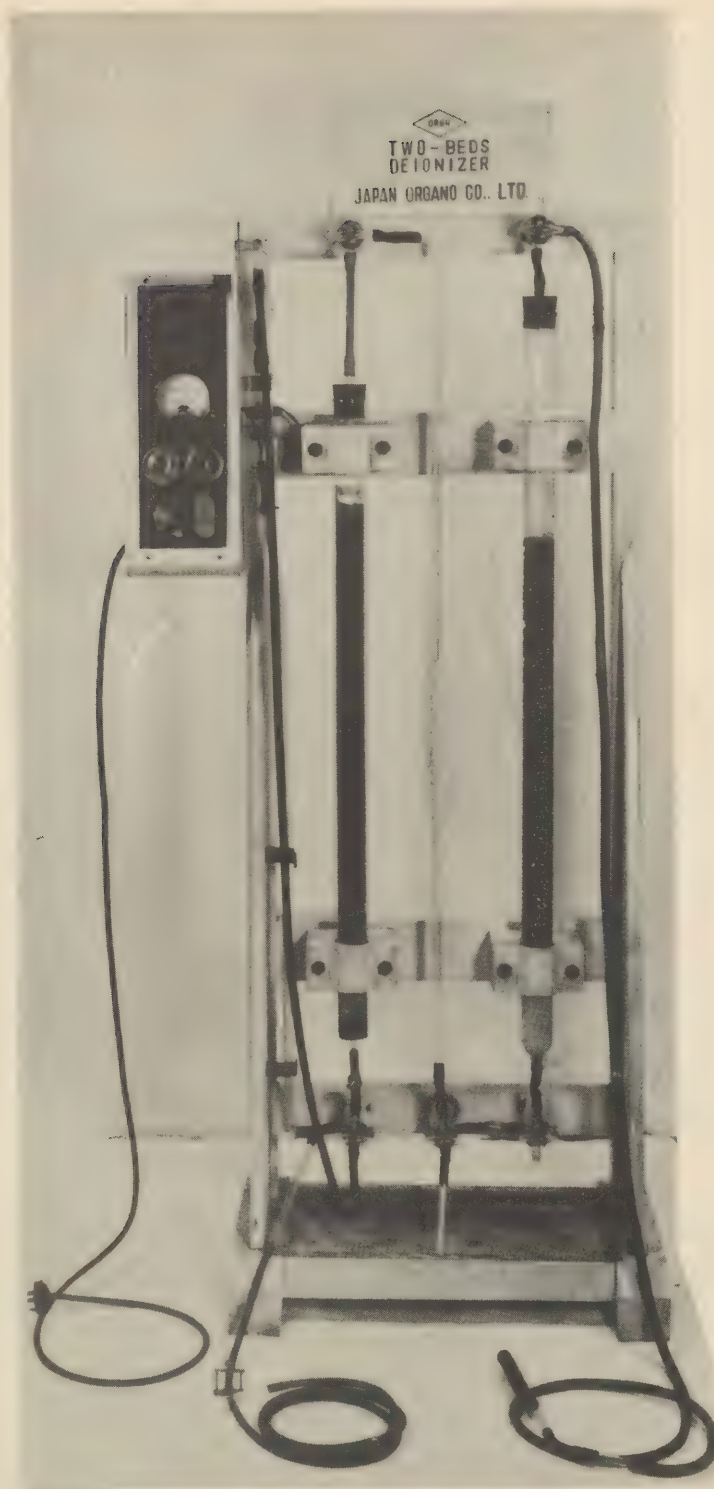
Figure 2. Double-Bed Deionizer

acid solution to pass through the cation resin in 30 minutes. In a similar manner, one liter of 5% base is passed through the anion resin in 30 minutes. The system is then rinsed with tap water for 30 minutes to wash out excess regenerant and the unit is ready to produce further deionized water. If the system stands inactive for an appreciable time such as overnight, it should be flushed with water prior to collection of water for use, since it has been found here that the first three or four gallons of water produced each morning are contaminated with dissolved solids. A quick examination for chlorides, by use of silver nitrate solution, is satisfactory since chlorides are high on the EMF series and give an indication which ions are being removed. If the tests for chlorides are negative, it has been shown that the water produced is satisfactory.

Rate of flow through the double-bed exchange system was regulated at 3.6 liters per hour for maximum efficiency using weights of resin described above. The resins used in the double-bed system were Rohm and Haas "Amberlite" (29) with IR 4B being selected for the cation and IRA400 as the anion exchange resins. The reason for this selection is that these resins were available in the Far East Command to the Organo Company, whose representatives agreed to make pilot models of the units to be examined at this laboratory.

The mono-bed or mixed-bed deionizing unit operates on the same theory as the double-bed type except that the two resins are thoroughly mixed in a single chamber (Fig. 3). This, in effect, gives an exchange column of high capacity since hydrogen and hydroxyl ions released do not have time to become stabilized with other ions in solution by forming such ions as bicarbonate, bisulfate, and other weak ions. Regeneration of the mono-bed system may be achieved by separating the two resins by reverse flushing with water on the basis of their different specific gravities. After the layers are separated, each layer is recharged with the appropriate regenerant, starting with the top layer and allowing the regenerant to pass through the entire column. The bottom layer is then recharged using a thistle tube arrangement which is lowered to the separation point of the resins, with care being taken not to allow this solution to contact the upper layer. Regeneration is at the same rate as for the double-bed system and the rinsing process with tap water is the same. The resins are remixed following rinsing by forcing a stream of compressed air from the bottom of the column which also contains sufficient water to facilitate mixing. After mixing, the column is ready for use once more.

A third type of exchange apparatus was designed with two anion and two cation exchange columns in series, (Fig. 4). This offers the advantage of compactness and produces a product of similar quality to that of the double-bed system.

Table XVI outlines the resins used in each unit. All resins are those of the Rohm and Haas Company.

Table XVI. Ion Exchange Units

Type of Unit	Cation Resin	Anion Resin
Single Bed	Strongly Acidic IR110	Strongly Basic IR400
Double Bed	Strongly Acidic IR4B	Strongly Basic IR400
Four Bed	Strongly Acidic IR4B	Strongly Basic IR400

Water collected from these various units was evaluated according to the procedures of U.S.P. XIII (28). A comparison of the results is shown in Table XVII.

Results of analysis demonstrates the mono-bed system to be the best for producing water of low total solids content. Since the three units examined use different resin components, it appears that the best resins for producing laboratory waters are the strongly acid and strongly basic resins.
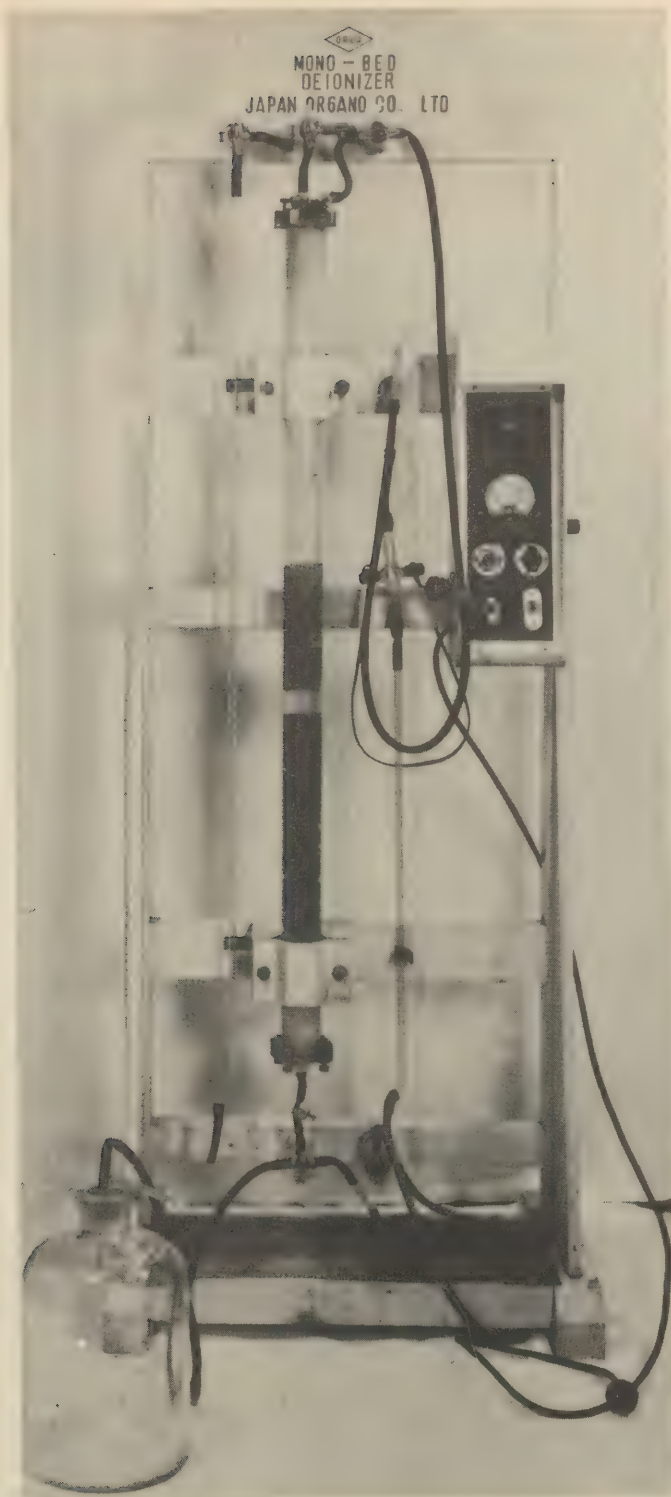
Figure 3. Mono-bed Deionizer
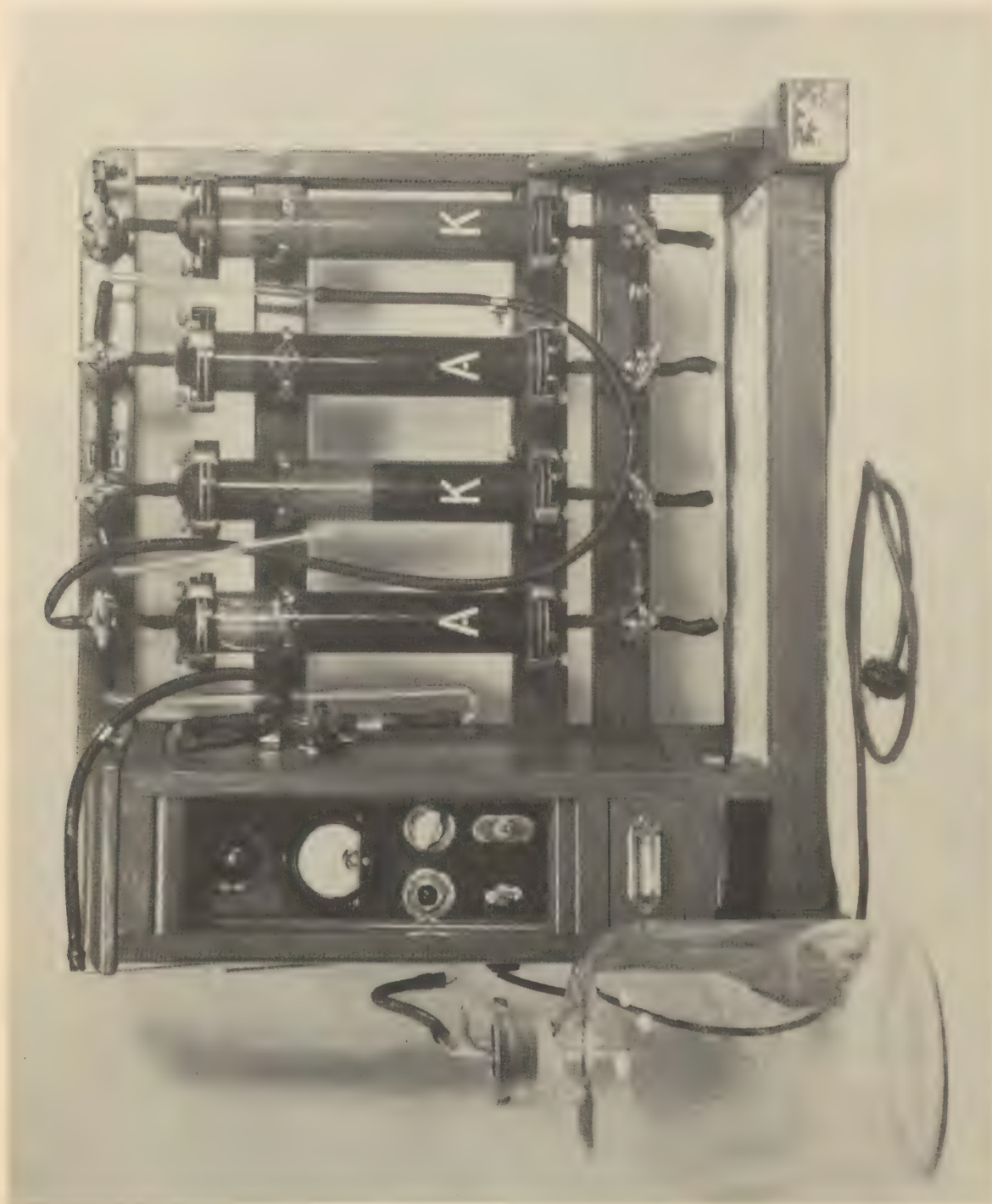
Figure 4. Multiple-bed Deionizer

Table XVII. Analysis of Deionized Water

Determination and Apparatus		1	2	3	4	5	6	7	8	9	10	11
		Sample (Five Gallon Aliquot)										
Chlorides	1	N	N	SP	N	SP	N	N	N	SP	SP	P
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Ammonia	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	SP	N	N	P	P	P	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	SP
Calcium	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Carbon Dioxide	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Oxidizing Substances	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Heavy Metals	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Sulfates	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Reaction (Methyl Red)	1	N	N	N	N	N	N	N	N	SP	P	P
	2	N	N	N	N	P	N	P	P	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
Reaction (Bromothymol Blue)	1	N	N	N	N	N	N	N	N	N	N	N
	2	N	N	N	N	N	N	N	N	N	N	N
	4	N	N	N	N	N	N	N	N	N	N	N
pH	1	6.7	6.5	6.4	6.3	6.3	6.3	6.2	6.2	6.2	6.2	6.1
	2	6.0	6.1	6.0	6.0	5.9	5.8	6.0	6.0	6.0	6.0	6.1
	4	6.0	5.9	6.1	6.3	6.3	6.2	6.0	5.9	6.0	6.2	6.2
Silica	1	0.8	0.2	0.6	0.6	0.6	0.6	0.4	0.4	0.4	0.4	5.0
	2	0.4	0.4	0.8	12	12	16	20	25	25	25	30
	4	6.0	8.0	8.0	10	8.0	12	10	10	8.0	6.0	8.0
Total Residue (ppm)	1	6	6	6	6	6	6	6	6	10	19	18
	2	6	8	10	14	18	20	22	28	28	30	31
	4	25	17	14	17	20	23	20	20	18	19	18

Fable: N - Negative, SP - Slight Positive, P - Positive

ALLERGY INVESTIGATION: Since the establishment of the Allergy Investigation Sec
tion in late 1950, various methods for collection of specimens, preservation, and ex-
traction of the various materials collected were tested, and the most suitable ones
were adopted as standard methods in this laboratory. A resume of work done by the
Allergy Investigation Section is shown in Tables XVIII and XIX.

Table XVIII. Specimens Received

Specimen	Number
Pollen slides received from 9 stations in Yokohama	2018
Plants collected for reference slides	453
Pollen collected for reference slides	453
Plates collected for mold counts	531
Materials for extraction	31
Total	3486

Table XIX. Examinations

Examinations	Number
Pollen slides examined	1200
Plants examined and classified	435
Pollen reference slides prepared	750
Plates examined	531
Fungus cultures from rice prepared	31
Fungus slides examined	36
Materials extracted (allergen preparation)	40
Total	3023

The investigation was carried out with emphasis on botanical surveys, atmospheric slide and plate collections, preparation of test extracts, and the collection of weather data.

Monthly botanical surveys were made of Yokohama and vicinity. These surveys consisted of a thorough check of the vegetation growing in the area at the time and a collection of the most typical plants present. These plants were examined and classified and extracts were prepared from the most common types, emphasizing the pollinating plants. Comparisons were made of the hayfever plants growing in the Tokyo and Yokohama areas as compared with those of the United States. From these studies a similitude of types of weeds was found in both Japanese areas and between Japan and the United States. In recent years, especially since the end of the war, the influx of foreign plants into Japan has accelerated and many new types of grasses and weeds have become firmly established in the port area. For instance, ragweed, the largest single causative agent of autumnal hayfever in the United States, is also found to be growing vigorously in the areas studied and in some locations has outweeded the native plants. This is contrary to some earlier reports (30, 31) which neglected the importance of ragweed in Japan as a hayfever plant. In addition to the typical hayfever plants found in the United States (various grasses, ragweed, thistles, dock, chenopods, plantain, birches, willows, privet, etc.) there are some indigenous types also. These include hops, mulberry, miscanthus, camanthus, Japanese cedars and others which have all the characteristics of being good hayfever plants.

During the winter, spores from molds, algae, fungi, mosses, and ferns must be considered as possible factors for allergies (32, 33, 34), since climatic conditions in Japan are such that some of these lower forms of plants are in their reproductive period when the air is very dry and there is little rainfall.

Rice, because of its abundance in Japan, is also considered a possible factor. Previous experimentation (35) has indicated its properties as an allergen. Seaweed is also considered (34, 36) since the cultivation and drying season of seaweed continues through the dry winter period when the wind acts as a vector for dissemination of the dried fragments.

Floral calendars and maps of hayfever plants of the Yokohama area have been made, pointing out the abundance of the plants found in the locality. Reference pollen slides and herbarium specimens were prepared from the different plants collected. Photographing of the pollen reference slides was started in establishing a file containing the data on all the common plants found in the areas under observation. Nearly 1200 plants were tabulated with information including place of growth, abundance, pollinating season, etc. This file, when completed, will facilitate the recognition of the various types of pollen found in the atmosphere.

Daily atmospheric slide collections were made by exposing slides, lightly coated with glycerin jelly (37), to the air for 24 hours at nine stations established at strategic points throughout Yokohama. These slides were examined and the many types of vegetation, molds, and other materials in the air were observed and recorded. Following is a list of the types of pollen and other substances noted in the greatest abundance during specific seasons:

SPRING .... Cedar   Willow   Acacia
                   Birch   Oak   Yew
                   Alder   Pine

SUMMER .... Plantain   Grasses (timothy, rice, etc.)
                   Dock   Molds
                   Sycamore

AUTUMN .... Hops   Pigweed
                   Ragweed   Wormseed
                   Goldenrod   Osmanthus
                   Miscanthus

WINTER .... Spores and fragments of various members of
                   Filinicae and Algae.
                   Fragments of stalks and leaves of rice plants.
                   Seaweed
                   Sawdust

Weekly atmospheric plate collections for molds (38) were made on Sabouraud's and rice extract media by exposing the sterile plates to the air for two minutes at each of nine stations. It is pointed out by Feinberg, Morrow and Lowe (34), and several others, that molds present in the air must be considered as a cause of allergic diseases. The molds most commonly found on the plates were: Alternaria, Aspergillus, Penicillium, Mucorsporium, and Microsporium.

Antigenic extracts were prepared for certain pollens and molds. The pollen was collected in large quantities during its respective season and was extracted by the method of Strauss and Spain (39) with some modifications. Pure cultures of the molds of interest were obtained and allergenic extracts prepared. Extracts of rice were prepared by methods described in Cooke (40). Extracts of house dust were also prepared for clinical testing. Samples of dust were collected from the houses of people who were affected with the asthmatic condition. The dust samples were extracted as described in Cooke (40) and Vaughan and Black (41). All extracts were sterilized for clinical testing by Seitz filtration and sterility tests were performed on each extract by the Bacteriology Department. All extracts, with the exception of dust extracts, were standardized by the determination of the protein nitrogen factor in the extract and converted into protein nitrogen units. The method as described in Cooke and in Vaughan and Black was used.

Yokohama Asthma - Late in 1950 the laboratory was asked to participate in an investigation of the causes of the disease known locally as "Yokohama Asthma". This condition was reported as being a health problem with American forces since 1946. Symptomatology ranges from mild bronchial irritation to severe respiratory distress

requiring emergency treatment. Patients fail to respond to the drugs used commonly
in treating allergic states and, with few exceptions, exhibit no previous history of
asthma or of being "allergic types". While there is some evidence that this condi-
tion is found in other areas of Japan, it appears to be a major problem particularly
in the immediate Yokohama area. In fact, patients are reported to often find quick
relief when they move even short distances from Yokohama.

Yokohama Asthma prevails for the period beginning with September or October and
continues through March or April. It is characterized by sudden outbreaks beginning
in the late evening or early morning hours. Occasional cases are seen throughout the
year. Whether or not these sporadic cases are actually Yokohama Asthma may be dispu-
ted; the non-specific nature of the symptoms do not permit any hard and fast diagnosis.

The overall picture does not appear compatible with allergic asthma for several
reasons: The asthma season occurs during the period when pollen counts are lowest;
antihistamine drugs are ineffective in relieving the disease; the patients do not
have a previous history of asthma; and the disease appears to be confined to or accen-
tuated in a relatively small area.

Some aspects of the disease suggest an air pollution problem similar to, but less
acute than, that which existed during the much publicized smog episode at Donora, Penn-
sylvania in October 1948 (42). The high industrialization of Yokohama and its location
on a bay inclosed by hills and bluffs offer ideal conditions for the formation and re-
tention of smog. Observation of the city from these hills often shows large sections
of the city to be obliterated by smog. If smog is an etiological factor, or one af-
fecting the course of Yokohama asthma, those weather conditions which tend to retain
smog in an area or which tend to dissipate it should affect the incidence and inten-
sity of the disease. Therefore, a systematic attempt was made to relate weather and
Yokohama Asthma. This was made possible through the efforts of the 155th Station Hos-
pital in Yokohama where asthma incidence data were recorded, and the Far East Air
Forces which submitted tabulated meteoroligic data obtained from a Japanese weather
station located in Yokohama. At present this laboratory has not obtained a complete
clinical evaluation of the Yokohama Asthma picture, and has had to be content with a
mere numerical listing of cases phoning or presenting themselves at the 155th Station
Hospital during the months of November and December, 1950, and September through Decem-
ber, 1951. No attempt has been made at this point to correlate various degrees of
severity of asthma with weather.

The following weather data were plotted against asthma incidence: wind velocity
and direction, barometric pressure, temperature, relative humidity, visibility, precip-
itation, and tide heights. Only wind velocity and precipitation appear to bear any re-
lationship to asthma incidence; hence, the data shown in Figures 5 through 10 are lim-
ited to these two weather observations. The daily wind velocities shown were obtained
by averaging the data collected at 0600; 1000, 1400, 1800 and 2200 hours.

These graphs reveal, in a general way, that it is on those days preceded by little
or no rainfall and where wind velocities are low that the greatest incidence of asthma
is found. Conversely, it is noted that a drop in incidence seems to follow a rainfall
or heavy wind. Because there are some notable exceptions to these generalizations, the
periods where high asthma incidence are recorded (arbitrarily, 10 or more cases) will
be discussed individually.

Throughout the period of November 12 through 17, 1950 (Fig. 5) there was no rain-
fall. Asthma incidence rose gradually from the beginning of this period until on the
14th there were eight cases. On the 15th, rather brisk winds were blowing (8.2 mps*).
On this day the incidence dropped sharply to two cases. On the following day, winds
were again mild (3.5 mps); the incidence climbed to 13 cases. On the 17th, the wind
velocity rose slightly (5.2 mps) and five cases were listed. A heavy rainfall on the
18th (38.5 mm) is associated with a drop in incidence to one case. Mild winds and

_____

* Meters per second; for a close approximation of knots multiply by two.

no rainfall were observed on the 19th and 20th, and again the asthma rate climbed rapidly, with 16 cases being listed. The decline from this incidence peak can again be related to increased wind velocity (6 mps) on the 21st and rainfall (6 mm) on the 22nd.

During December, 1950 (Fig. 6) high asthma incidence peaks were seen on the 7th, 13th, and 30th. From the 2nd through the 7th, no rain fell in Yokohama; from 9.8 mps on the 2nd, the average wind velocity gradually declined to 2.3 mps. Concomitantly, asthma incidence rose to nine cases. Relatively strong winds on the 6th seemed to check the incidence rise temporarily, but on the 7th (wind velocity 1.2 mps) the asthma rate climbed to an alarming 21 cases. Rainfall and increasing wind velocities on the 8th and 9th coincided with a rapid decline in asthma incidence. The period from the 10th through the 13th was characterized by mild winds and the absence of rain. It is also characterized by the rising asthma incidence rate with a maximum being reached on the 13th (14 cases). On the 14th there was a small rainfall (4 mm); asthma incidence fell off sharply. After a drought of eight days (but with winds reasonably high), the incidence climbed to 11 on the 30th. That and the preceding day were marked by low wind velocities. The incidence curve dropped after a rainfall on the 30th.

Figure 7 shows two high asthma incidence peaks for the month of September 1951. The first of these is on the 24th, when 22 cases were reported. Low wind velocities (less than 4 mps) had been observed for three days previously, and rainfall was sparse, with only a small amount of rain (3 mm) falling on the 22nd. A rapid drop in incidence on the 25th coincided with heavy precipitation that day (37 mm). Low wind velocities and no precipitation on the 28th and 29th seemed to result in high asthma incidence on the 30th. Rain on that and the next day was followed by a drop in incidence to zero.

Four high asthma incidence peaks are seen in October, 1951 (Fig. 8) on the 3rd (18 cases), the 7th and 8th (19 cases), the 12th and 13th (39 and 34 cases) and on the 17th and 18th (17 cases). As in the previous instances, all of these peaks are associated with low precipitation and low wind velocities. Similarly, as in the past, rapid declines in incidence coincide with high winds and precipitation, except for the last incidence peak (17th and 18th). Here asthma incidence fell off, even though there was no change in the precipitation-wind velocity picture. In view of previous experience, there is no ready explanation for this exception. The question is raised as to whether there are other factors than weather which might influence the cessation of asthma. It may also mean that a seizure of asthma "runs its course", terminating without a change in environment. Critical examination of detailed clinical reports might lead to a better understanding of such anomalies.

The incidence curve for November (Figure 9) shows four major peaks occurring on the 1st, 9th, 11th, and 20th. These follow the usual incidence-weather pattern but with exceptions. The incidence rate rises from one case on the 7th to 13 on the 9th, as would be expected from the low wind velocity and precipitation. However, the incidence drops inexplicably on the 10th to seven cases, only to rise again on the following day to 22 cases. Perhaps this deviation merely represents variation in what was generally an upward trend. There is a dramatic drop in incidence to zero on the 12th, even though there is not a great increase in wind velocity (from 2.9 to 6.2 mps) and the rainfall was light (1.3 mm). It should be remarked, however, that while precipitation was minimal, it was a long steady rain falling over a period of 15 hours. The quality of rainfall may be as much a factor as the quantity.

Only one major incidence peak was observed in December (Figure 10), with 21 cases on the 22nd and 34 cases on the 23rd. The 22nd was preceded by eight dry days and by three days in which the wind did not exceed 2 mps. A small rainfall (4.5 mm) on the 23rd (also an increase of wind velocity to 4.3 mps) was followed by a drop in incidence to 5 cases.

While more data is needed to definitely establish a correlation between wind velocity and precipitation and the occurrence of Yokohama Asthma, the prepared charts strongly indicate that a higher incidence of this respiratory disorder occurs when there
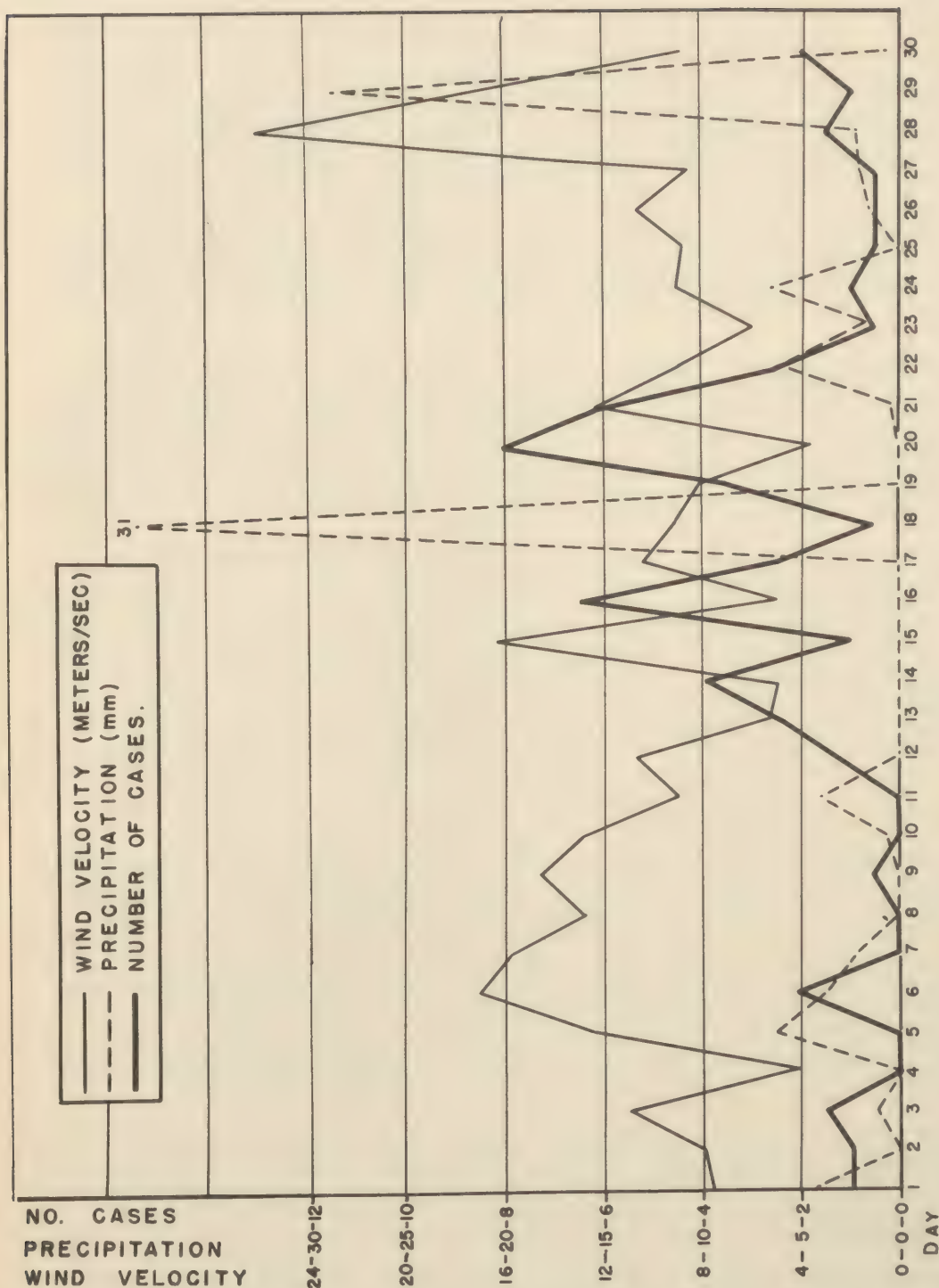
Figure 5. Yokohama Asthma, November 1950
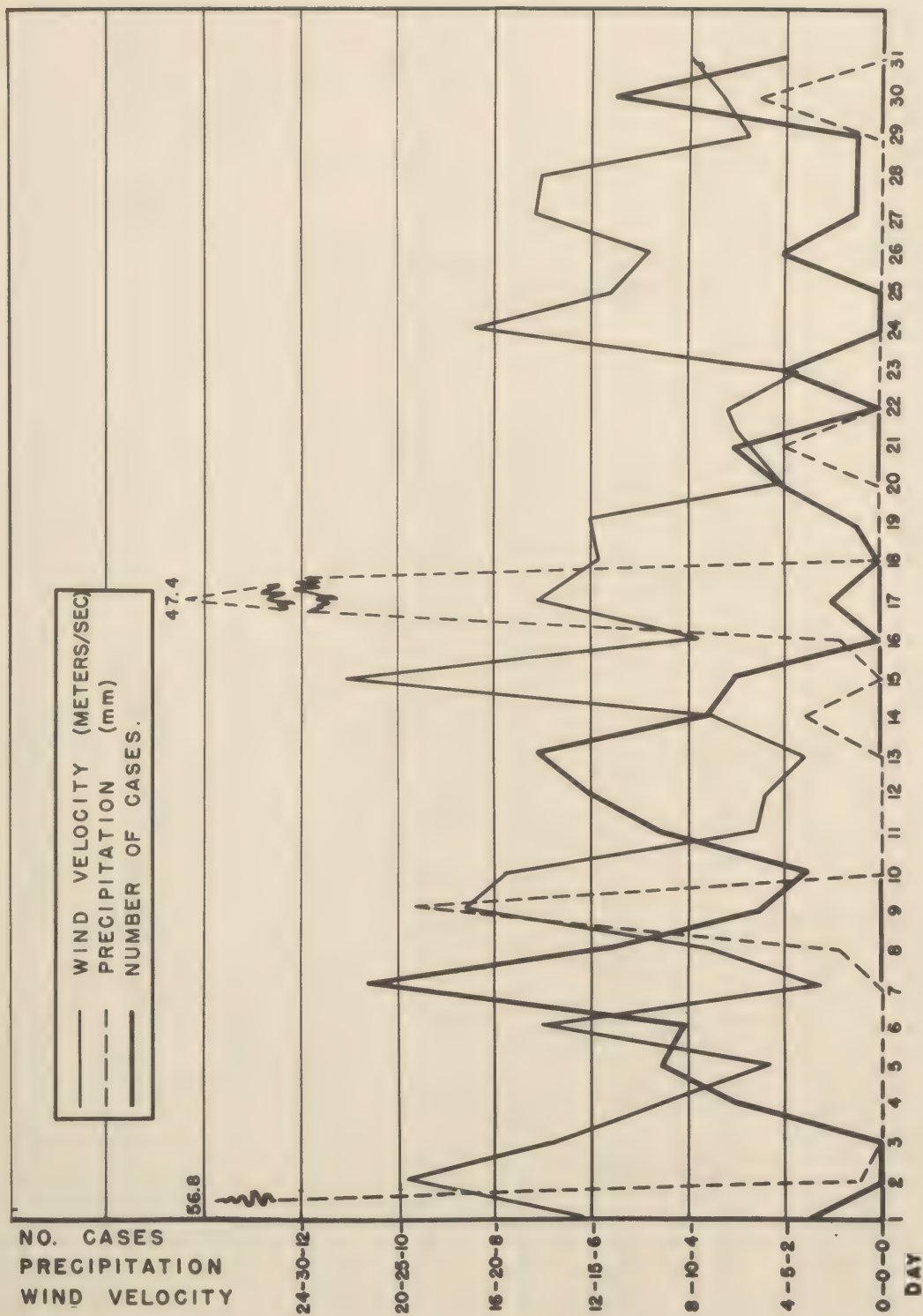
Figure 6. Yokohama Asthma, December 1950
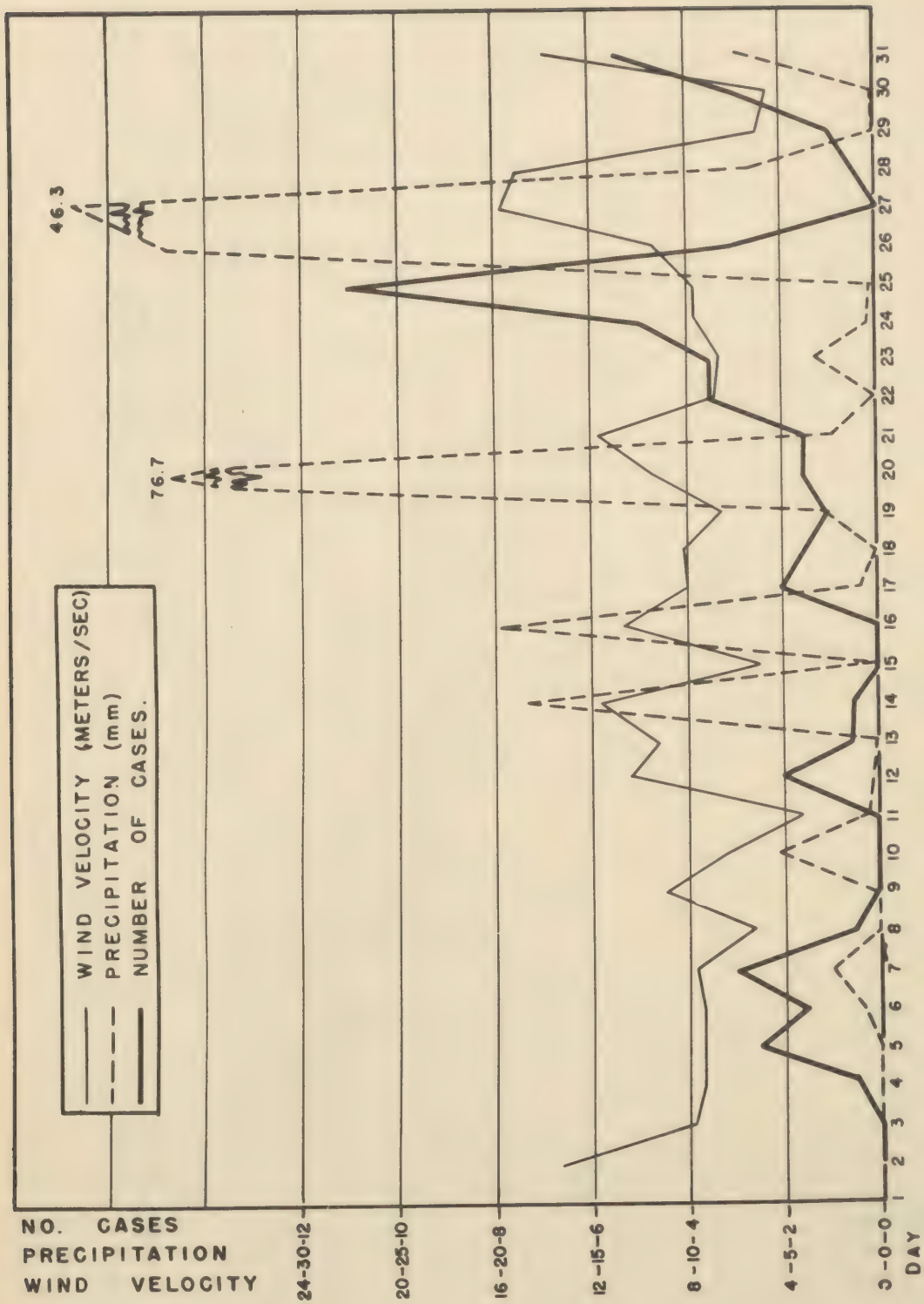
Figure 7. Yokohama Asthma, September 1951

Figure 8. Yokohama Asthma, October 1951
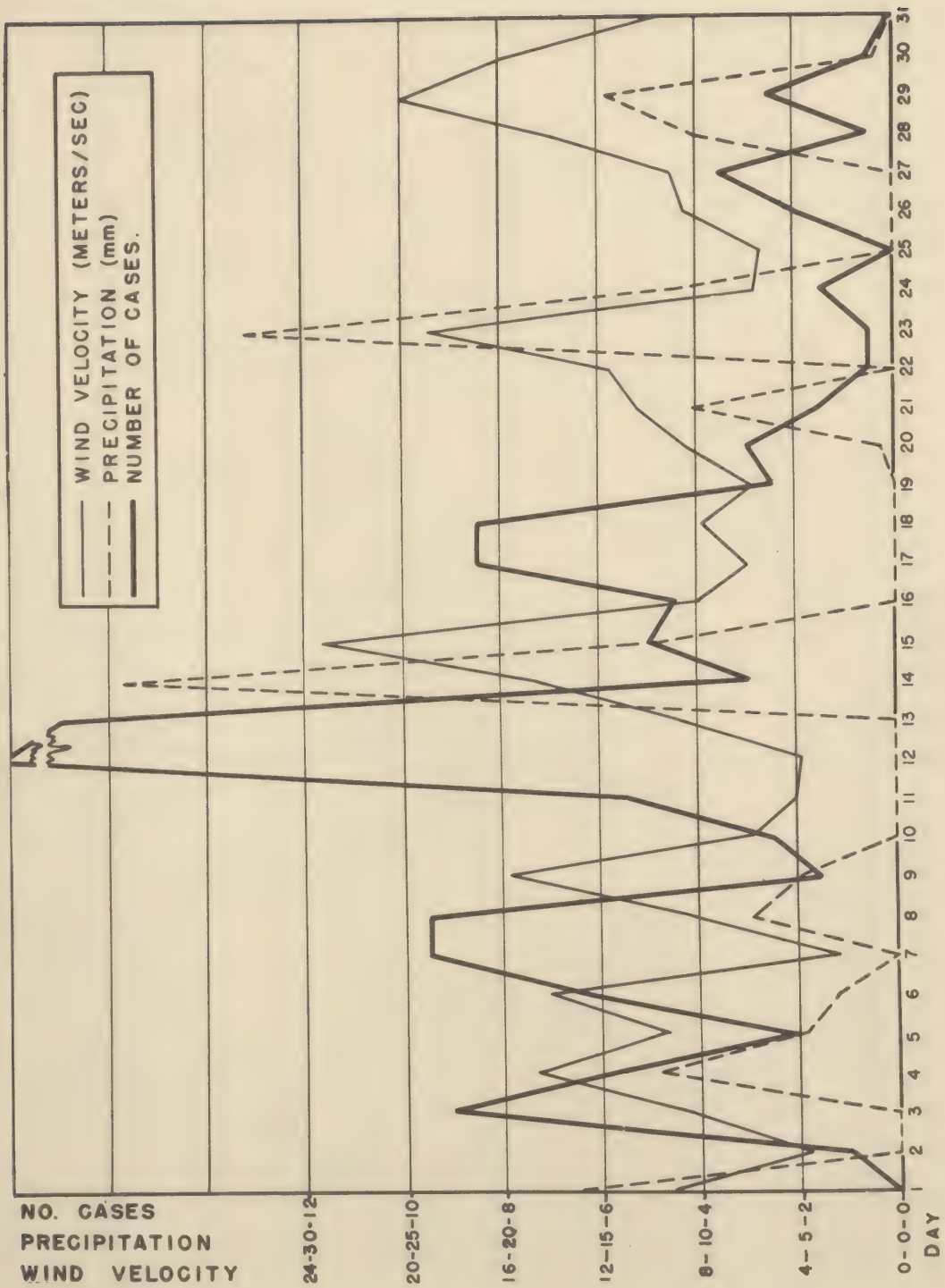
NO. CASES
PRECIPITATION
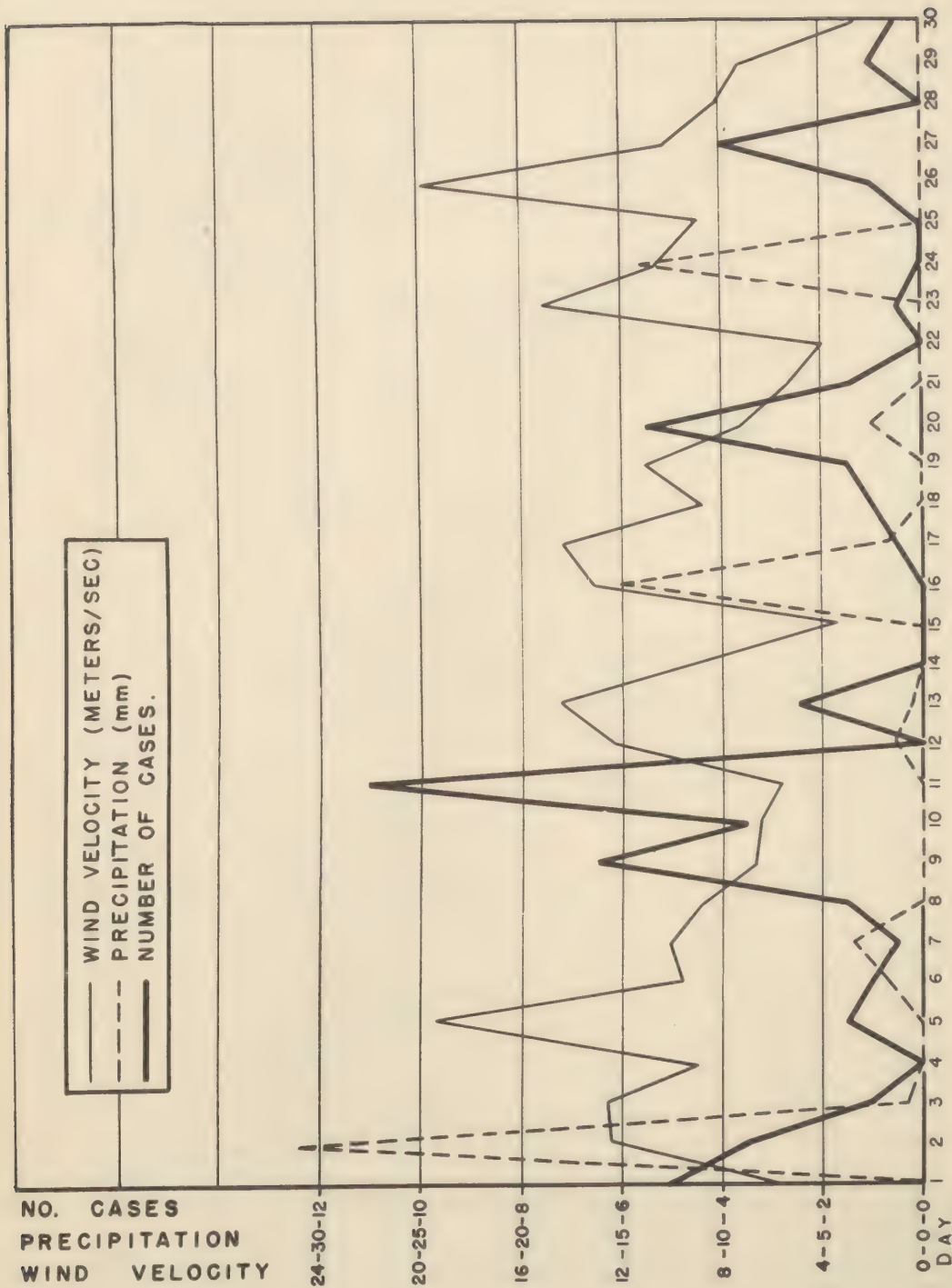WIND VELOCITY

24-30-12
20-25-10
16-20-8
12-15-6
8-10-4
4-5-2
0-0-0

DAY

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

WIND VELOCITY (METERS/SEC)
PRECIPITATION (mm)
NUMBER OF CASES.

- 191 -

Figure 9. Yokohama Asthma, November 1951
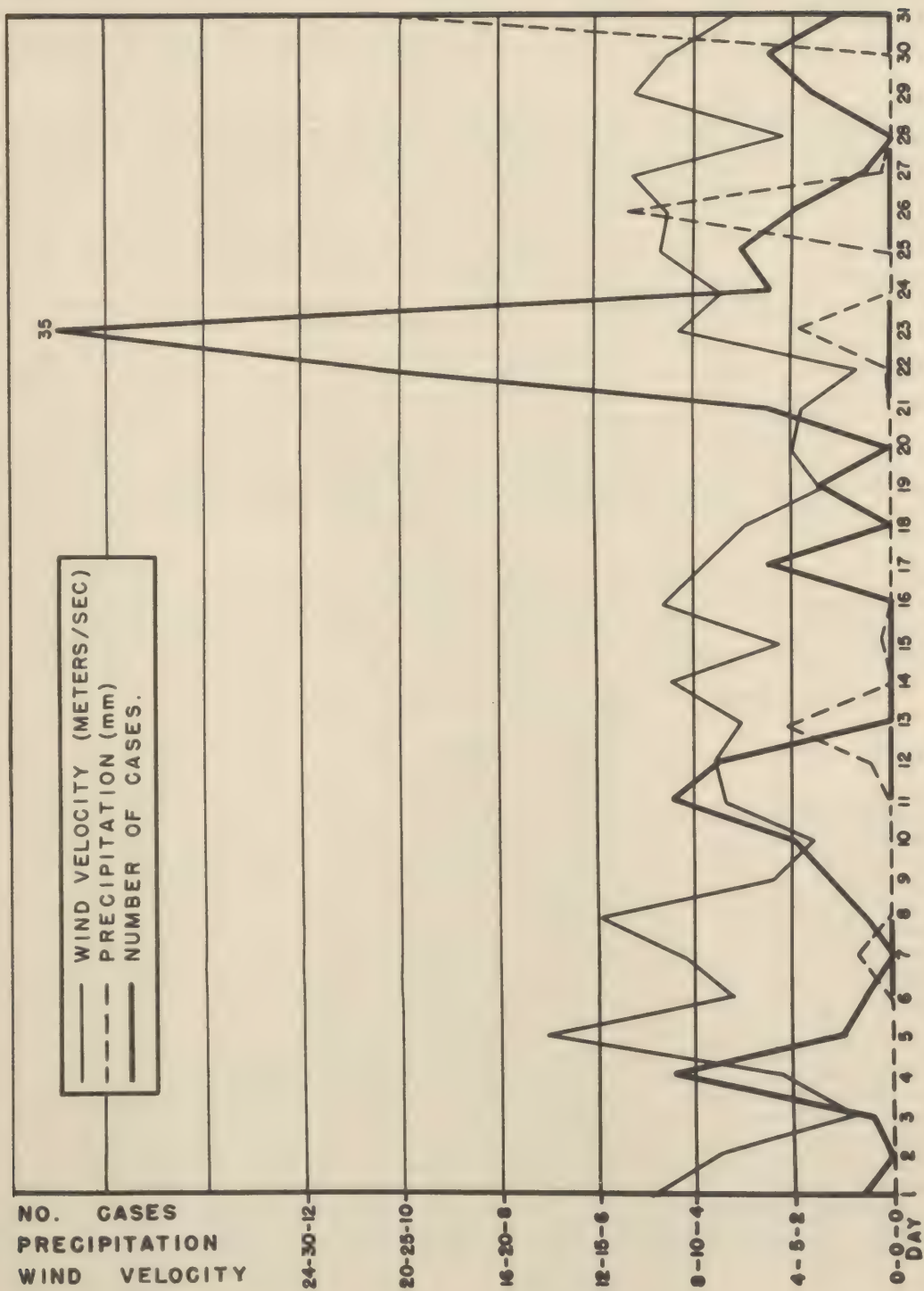
Figure 10. Yokohama Asthma, December 1951

has been little or no rain accompanied by winds of low velocity. If Yokohama Asthma is being caused by smog, this would be the expected finding. However, weather data of the type given can only supply indirect or supporting evidence in any study implicating smog as an etiological factor affecting the occurrence of asthma. Direct quantitative measurement of the elements that make up smog, coupled with a detailed, critical examination of the clinical data, would undoubtedly throw much light on the problem.

MISCELLANEOUS ANALYSIS: The Department of Chemistry has received numerous requests for analyses which generally are not easily classified as fitting into the operations of the other sections of the department. Many of the requests originate from military units other than medical, within the Far East Command, which have no laboratory or chemical analysis facilities available. However, the majority of these problems have been acute and of sufficient importance for this laboratory to make an attempt to produce an answer. One of the major difficulties confronting several of the analyses was lack of proper reagents or equipment to properly complete the work. Improvisation of equipment has been a test for the ingenuity of the technicians, and some working arrangement has been devised for each requirement. Purchase of non-standard reagents for analyses was, for a time, made from personal funds when regular supply procedures for procurement of non-standard items proved inexpedient, but this developed into too great a burden. The problem was presented to the Procurement Section of Japan Logistical Command whose officers examined the situation and provided a means for making emergency purchases. An authorization is now possible, upon approval of a representative of the Commanding Officer, to allow direct and immediate purchase of high quality reagent chemicals from indigenous sources and also to provide for minor items of equipment. This simple procedure has proved to be of the largest single aid in accomplishing special analyses expeditiously.

Table XX lists the variety of specimens received by the Department of Chemistry which have been referred to the Miscellaneous Analysis Section for examination. Some of these examinations have adapted themselves to existing facilities and procedures, but others required development of procedures or special modifications of techniques.

Table XX. Miscellaneous Specimens Received

Sample	No.	Sample	No.
Alcohol	88	Methanol	2
Alcoholic Beverages	128	Morphine Sulfate	14
Atomizer, Dental	2	Oil, Neatsfoot	5
BAL Ointment	20	Oxygen	411
Body Fluids	16	Pepsi Cola	1
Calcium Hypochlorite	31	pH Kit	1
Cigarettes	1	pH Standards	5
Coca Cola	1	Phenol Solution	1
Cresol	16	Pontocaine	30
Dextrose	3	Procaine, Crystaline	3
DDT Powder	77	Procaine Solution	158
Diacs (Heat Indicators)	4	Prophylactics, Mechanical	192
Dye, Linen	1	Shell Fragment	1
First Aid Kit, Korean	1	Soap, Laundry	21
Frostbite Solution	370	Soil	1
Gastric Contents	1	Sucrose	1
Germicide Solution	1	Thromboplastin	36
Halazone Tablets	291	Unknown Substances	106
Ion Exchange Apparatus	1	Water	7
Insulin, Protamine Zinc	10	Water Purification Tablets.	30
Liver Injection	6	Total	2095

The variety of determinations accomplished by the Miscellaneous Analysis Section has required extensive use of technical literature and has demonstrated a need for any laboratory of this type having available for use good standard texts for qualitative analysis of both organic and inorganic compounds. Those books which have been found most useful to this laboratory are Feigl (12), Huntress and Mulliken (43), United States Pharmacopoeia (the more editions the better), New and Non-Official Remedies (44), Modern Drug Encyclopedia (45), Goodman and Gilman (46), National Formulary (47), and a number of standard texts on qualitative and quantitative chemistry. In addition to having this source of information, it is necessary to have full quality specifications available in any case where quality control and quantitative analysis are required. In some cases, this laboratory has been requested to assist in writing specifications for procurement of chemical reagents of various types.

Table XXI demonstrates the variety of examinations required for completion of the various requests submitted.

Table XXI. Determinations Completed by Miscellaneous Analysis

Qualitative	No.	Qualitative	No.
Acetaldehyde	1	Chromatographic Analysis	4
Acetanalid	3	Citric Acid	1
Acetone	6	Cocaine	1
Acids	84	Codeine	1
Alcohol, Ethyl	5	Copper	1
Alcohol, Isopropyl	2	Corrosion Tests	47
Alcohol, Methyl	135	Cotton Fiber	1
Aldehydes	127	Cresol	1
Alkalies	103	Cyanide	107
Alkaloids	178	Cystine	1
Aluminum	9	DDT	5
Aluminum Oxide	4	Dextrose	1
Aluminum Silicate	1	Dimercaprol	20
Amides	1	Disazo Dye	6
Amino Acids	2	Dramamine	2
Ammonia	4	Fatty Acids	1
Aniline	1	Fish Poisons	3
Arsenic	2	Formaldehyde	1
Artificial Coloring	3	Fusel Oil	109
Aspirin	9	Glycerine	1
Atabrine	6	Gum Tragacanth	1
Atropine	2	Halazone	5
Barbiturates	66	Halogenated Compounds	93
Benadryl	3	Hashish	1
Benzedrine	31	Heavy Metals	113
Bicarbonate	1	Heparin	1
Boric Acid	1	Heroin	27
Borneol	6	Hydrogen Peroxide	1
Bromides	1	Hydroxide	1
Cadium	3	Inorganic Substances	1
Caffeine	5	Iodine	1
Calcium	3	Iron	2
Calcium Carbonate	3	Kerosene	4
Calcium Glycerophosphate	5	Ketones	109
Calcium Hypochlorite	2	Lanolin	2
Calcium Oxide	1	Licorice	1
Camphor	1	Magnesium	1
Carbonates	6	Magnesium Oxide	2
Carbon Dioxide	93	Magnesium Silicate	1
Carbonizable Matter	1	Magnesium Stearate	1
Carbon Monoxide	93	Marihuana	4
Chloral Hydrate	106	Menthol	1
Chloramine T	1	Mercury	1
Chlorides	3	Mercury Phenylacetate	1
Chlorine	2	Merthiolate	1
Chloroform	2	Mineral Acids	2
Chloroquine Phosphate	2	Morphine	49
Chlorosulfonic Acid	5	Neatsfoot Oil	1

Table XXI. Determinations Completed by Miscellaneous Analysis Continued

Qualitative	No.	Qualitative	No.
Nicotine	2	Silicon Dioxide	1
Novocaine	1	Soap, Anhydrous	5
Opium	7	Soda Ash	1
Organic Matter	27	Soda Soap	3
Orthophenyl Phenol	2	Sodium	7
Oxalate	1	Sodium Bicarbonate	12
Oxidizing Substances	79	Sodium Carbonate	14
Paraformaldehyde	1	Sodium Chloride	1
Penicillin	1	Sodium Fusion	1
Petrolatum	1	Sodium Oxide	1
Phenacetin	1	Sodium Silicate	1
Phenobarbital	1	Spectrographic Analysis	8
Phenol	95	Starch	18
Phosphates	1	Sulfadiazine	1
Pontocaine	6	Sulfates	1
Potassium	3	Sulfathiazole	6
Potassium Hydroxide	1	Sulfonamides	1
Potassium Iodide	2	Sulfur	1
Potassium Oxide	1	Tin	1
Procaine	6	Tobacco	1
Protein	2	Tridione	1
Pumice	1	Ureides	1
Reducing Substances	1	Uric Acid	1
Salicylates	1	Water, Distilled	1
Saponin	1	Wellman's Test	1
Schonvogel's Test	1	Yeast Cells	1
Secobarbital	2	Zinc	1
Seconal	5	Zinc Oxide	1
Silica	2	Zinc Stearate	1
Silicates	7		

Total 2230

Quantitative	No.	Quantitative	No.
Acetone	1	Chlorine	350
Acid, Free	13	Cresol Assay	8
Acidity	53	DDT Assay	78
Alcohol, Ethyl	417	DDT, p.p.	2
Alcohol Insoluble Matter	18	DDT, o.p.	2
Alcohol, Methyl	68	Dextrose	210
Aldehydes	41	Dimercaprol	20
Alkali, Free	21	Distillation	44
Arsenic	2	Heavy Metals	3
Barbiturates	4	Insulin Assay	1
Benzene	4	Iodine, Free	30
Boric Acid	2	Iodine Number	7
Carbonizable Matter	1	Lead	2
Calcium Oxide	2	Matter Volatile at 105 C	18
Chlorides	15	Moisture	6

Table XXI. Determinations Completed by Miscellaneous Analysis Continued

Quantitative (Cont'd)	No.	Quantitative (Cont'd)	No.
Morphine	2	Residue on Ignition	4
Non-Volatile Residue	50	Resin	18
Organic Impurities	22	Soap, Anhydrous	18
Orthophenyl Phenol	1	Sodium Chloride	2
Oxygen Assay	431	Total Residue	2
Paraformaldehyde	2	Unsaponifiable Matter	3
Particle Size, 80 Mesh	50	Vegetable Oils	2
Particle Size, 100 Mesh	50	Vitamin B-12 Assay	6
Particle Size, 325 Mesh	50	Water	43
Phenol	1	Water Insoluble Matter	18
Pontocaine Assay	6	Weight Loss on Ignition	3
Procaine Assay	318	Zinc	1
		Total	2546

Physical Constants	No.	Physical Constants	No.
Acidity	1	Odor	37
Alkalinity	1	pH Determinations	17
Appearance	58	Platinum Fusion	1
Boiling Point	2	Reaction	5
Color	57	Refractive Index	7
Distallation	14	Solubility	35
Melting Point	27	Specific Gravity	55
Miscibility with Water	42	Water Emulsification	1
		Total	360

Miscellaneous	No.	Miscellaneous	No.
Diatom Identification	2	Physical Measurements	144
Equipment Check	4	Rabbit Injection, Insulin	24
Inflation Check	96	Systematic Qualitative Analysis	3
Microscopic Examinations	6	Taxonomy Identifications	3
Mouse Injection, Insulin	200	Toxicity Tests, Animal	31
		Total	513

Total Miscellaneous Analyses 5649

        A total of 106 unknowns requiring a wide variety of chemical examinations were
received for analysis. One of the principal difficulties in identifying the unknown
samples has been the small amount of substance submitted and the lack of accompanying
information concerning the circumstances of apprehension or collection of the specimen.
Requests giving full details of the reasons for requesting analysis and other infor-
mation concerning the sample are much to be desired in order to expedite examinations.
Since the identification of chemical compounds entails considerable time, effort, and
expense, those samples which are submitted without cogent reasons for analysis are
held in abeyance until such information is available. In addition to the use of chem-
ical examinations the use of laboratory animals for establishing toxicity of specimens
has been invaluable and has been adopted as standard procedure at this laboratory in
any case where toxicity is considered a factor.

Of the 106 unknown specimens worked on, only four could not be positively identified. In the other 102 cases either positive identification was made or the full request was complied with short of complete and full identification procedures. Some of the identifications include the following: Heroin (one sample being 95 grams in weight), opium, nococaine, benadryl, sodium salicylates, hydrogen peroxide, "Health Maintaining Pills" of Japanese Imperial Veterinary Corps which were found to contain camphor-sugar-gum acacia, seed disinfectant, calcium hypochlorite, mislabeled chemicals, buckwheat husks, Rhus verniciflua (poison sumac), opium cigarettes, coffee extract, Cannabis indica (hashish), chloroquine phosphate, secobarbital, aspirin in cigarette, DDT, chlorosulfonic acid, Cannabis sativa (marihuana), methyl propamine, sediment from jet engine, and others.

One identification was requested by the Quartermaster, GHQ, concerning embalming powder which was desired to be purchased from local markets. Qualitative analysis of the sample showed the presence of lime and paraformaldehyde. A procedure was set up for paraformaldehyde assay, based on U.S. Dispensary (48), and for calcium oxide to furnish the necessary information for procurement.

The Miscellaneous Analysis Section has received a variety of requests for examinations other than those for identification only. These projects have included the assay of a number of products which were procured from indigenous sources and for which additional measures of quality control were required either to insure uniformity of the product furnished or to assure a margin of safety for the use of the product. Some of the projects have been carried over from previous years and are more or less routine. Examples of the diversity of substances to which such examination pertain are: Alcoholic beverages, laundry soap, denatured alcohol, water purification tablets, insulin, BAL ointment, cresol, pH kits, dental atomizers, shell fragments, soil samples, fish poisons, pontocaine and procaine solutions, vitamin B-12, PX film processing solutions, DDT, and mechanical prophylactics.

Determination of Procaine in Frostbite Solutions - The laboratory receives occasional requests for procaine determinations where solutions are being questioned because of their real or imagined pharmacologic ineffectiveness. Since, at the time, no good U.S.P. method of analysis existed for this anesthetic, a method of assay for procaine was studied at this laboratory (49). This method is based on the Bratton-Marshall determination of sulfonamides (50) and depends upon the diazotization of a primary aromatic amine followed by coupling with N-(1-naphtyl)-ethylene-diamine to form a dye. It was recognized at the time that, as in most chemical assays, only part of the molecule is being measured - in this case, that end of the procaine molecule harboring the primary aromatic amino group - and thus, the method does not insure that the final result represents intact procaine. On hydrolytic degeneration, procaine yields p-amino benzoic acid and diethylaminoethanol. Because the method measures the p-amino benzoic acid portion of the molecule, it does not differentiate between the intact and hydrolized procaine. Nevertheless, because of the relative stability of sealed, sterile procaine solutions, it was felt that the concern over instability was unwarranted, and that if the solutions were not actually discolored, they had not degenerated appreciably. As will be shown, this assumption was correct.

Late in 1950, this laboratory was called upon to establish specifications for and to control the quantity of procaine in a solution to be used in treatment of frost bite being prepared for the Armed Forces in large quantity by a local firm. Emergency conditions existed at the time, and, from the chemical viewpoint, the expedient of insuring adequate control of the specified quantities of glucose (5%), ethanol (5%) and procaine hydrochloride (0.1%) in the finished product was of primary concern. The above method was employed in control analyses. Since then, a method utilizing alkaline chloroform extraction has appeared (51). This year more detailed study of the stability of the procaine solution has been performed utilizing this newer method of assay. It appears that in the presence of glucose, the content of intact procaine in the solution decreases rapidly (Figure 11). Decreasing the temperature greatly retards the reaction (Figure 12).

soln. A. = Glucose (5%),
    alcohol (5%), procaine HCL
    (0.1%).

soln. B. = Glucose (5%)
    procaine HCL (0.1%)

soln. C. = Alcohol (5%)
    procaine HCL (0.1%)
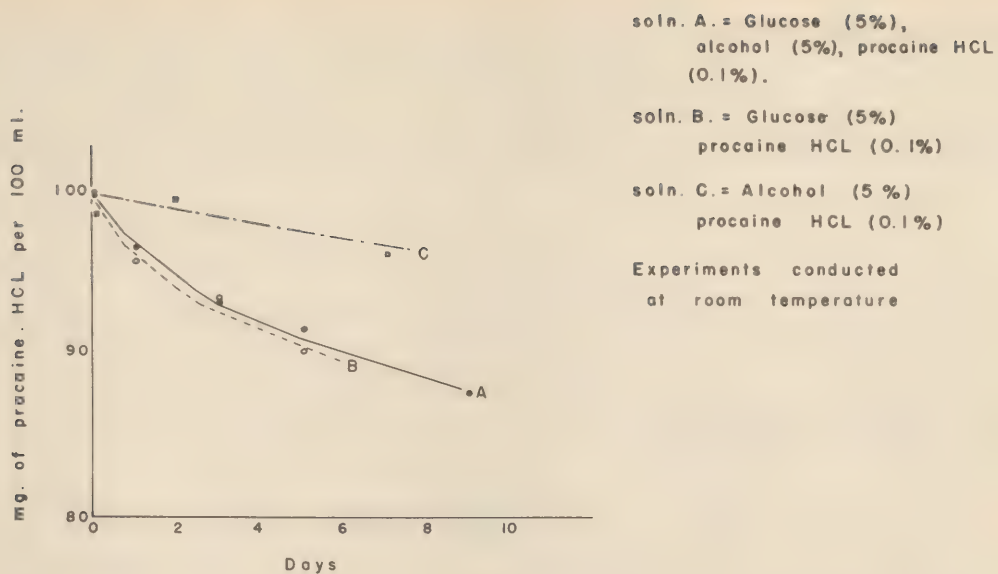
Experiments conducted
at room temperature

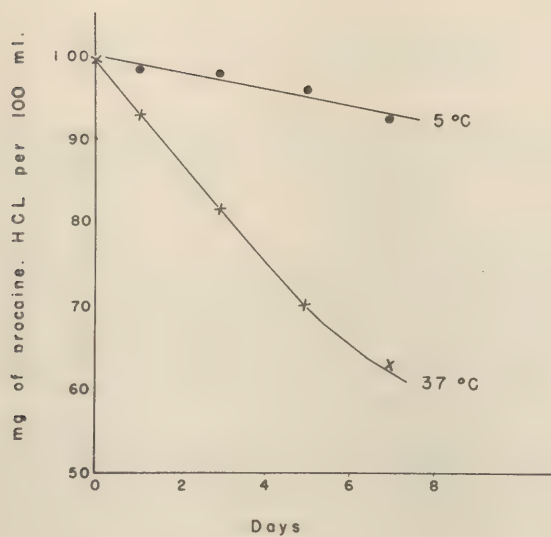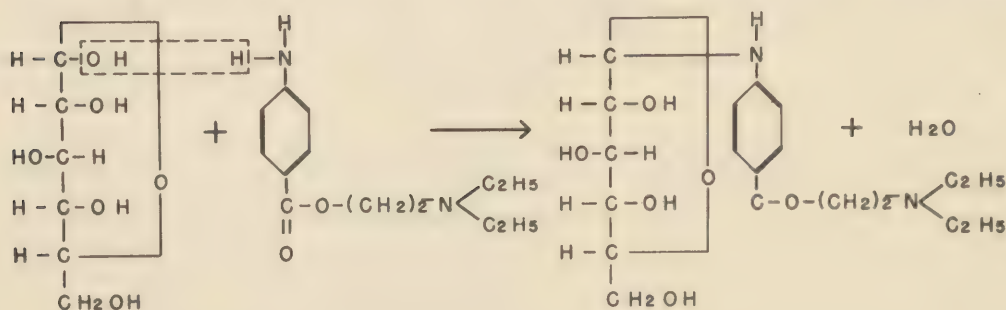Figure 11. Disappearance of Free Procaine in Procaine-Glucose Solutions

Figure 12. Effect of Temperature on the Disappearance of Free
Procaine in Procaine-Glucose Solutions

The low procaine values can probably best be accounted for by the following
reaction:

GLUCOSE + PROCAINE $\longrightarrow$ PROCAINE GLUCOSIDE + WATER

In order to determine whether the procaine was being destroyed or was combining
as indicated above, frostbite solutions were subjected to paper chromatography. Com-
mercial procaine solutions, suspected by the dental clinic of having degenerated, were
tested in the same manner.

Principle - Partition chromatography differs from adsorption chromatography
in that separations depend upon differences in partition of a solute between two im-
miscible or partially immiscible liquid phases, one mobile and the other stationary,
rather than upon the adsorptive properties of a solid stationary phase. The paper
partition chromatographic technique was instigated (or at least rediscovered) by Con-
sden, Gordon and Martin (52), and while there has been much elaboration and modifica-
tion of the original technique, it consists essentially of the following: A filter
paper strip, the upper end of which is immersed in a trough containing the water-
saturated solvent, hangs in an airtight chamber in which an atmosphere saturated with
water and solvent is maintained. Before introducing the strip into the apparatus, a
drop containing the material to be chromatographed is dried on one end of the strip
and it is this end which is placed in the trough in such a manner that the dried mat-
erial does not touch the liquid of the trough. As the liquid descends the strip by
capillary action, the material migrates at a rate which depends upon its relative sol-
ubility (partition coefficient) in the stationary phase, represented by the water bound
in the cellulose, and in the mobile phase, represented by the descending solvent. The
position this material occupies with relation to the advancing solvent will be specific
for this material under a given set of conditions and is referred to as the R_f value.

The R$_f$ value may be defined mathematically as the movement of the solute divided by the movement of the advancing front of liquid.

In the following studies, the ascension technique of Williams and Kirby (53) as modified by Munier and Macheboeuf (54) was employed. Here the solvent <u>rises</u> in the paper by capillary action.

<u>Reagents and Materials</u> -

1. Hydrochloric acid, approximately 4 N.

2. Sodium nitrite, 0.1% solution.

3. Ammonium sulfamate, 0.5% solution.

4. N-(1-naphthyl)-ethylene-diamine dihydrochloride, 0.1% solution.

5. Standard procaine hydrochloride, 0.1% solution.

6. Standard p-amino benzoic acid, 0.1% solution.

7. Acetic acid-butanol solvent and aqueous phases: 100 ml butanol, 10 ml glacial acetic acid, and 50 ml water are shaken together thoroughly and allowed to stand for three days at room temperature before being separated into the solvent and aqueous phases. The three-day wait enables the solution to come to equilibrium with respect to the formation of butylacetate.

8. Ammonium hydroxide-butanol solvent and aqueous phases: This is prepared as in 7 above except that concentrated ammonium hydroxide is substituted for glacial acetic acid.

9. Neutral butanol solvent and aqueous phases: This is prepared as in 7 above except that additional water is substituted for glacial acetic acid.

10. Filter paper, 28 cm.^{2} sheets of Japanese manufactured filter paper (Toyo No. 2, Toyo Roshi Company)*.

<u>Procedure</u> - The procedure described by Munier and Macherboeuf (54) in their study of the chromatography of alkaloids is essentially the one employed here. A pencil line was drawn 4 cm from and parallel to one edge of the square filter paper. On points along this line at 3 cm intervals, 0.01 ml drops of the samples to be chromatographed were carefully placed using 0.1 ml Mohr pipettes. The samples were diluted so that 0.01 ml contained about 10 micrograms of procaine hydrochloride or p-amino benzoic acid. The paper was dried at room temperature. The paper was then bent perpendicularly to the pencil line into a cylinder and fastened at the end opposite the pencil line with paper clips using a paper bridge to separate the edges which closed the cylinder. The upright paper cylinder was then placed sample side downward into a Smillie jar, the bottom of which was covered with the solvent phase to a depth of

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* This is not the paper of choice for chromatographic studies, but was the only kind available at the time. The solvent migrates too rapidly, resulting in diffuse spots, and uniformity among sheets is lacking. More recently a comparison has been made between the following papers: Whatman No. 1, Toyo No. 50, Toyo No. 2, S&S No. 595.Their acceptability for chromatographic work is listed in the order given, with Whatman No. 1 being preferred. The rates of solvent migration were in their inverse order, respectively: 18.6 cm in 9.5 hours, 23.8 cm in 9.5 hours, 22.5 cm in 5.5 hours and 21.3 cm in 5.0 hours.

about 1 cm. A 50 ml. beaker containing a few milliliters of the aqueous phase was placed in the bottom of the jar in the area enclosed by the paper cylinder. The Smillie jar was closed, using stopcock grease to insure an airtight seal. The solvent was permitted to rise to about 20 cm. above the starting line. This required about five hours, with about half the distance being traversed during the first 90 minutes. The temperature during this series of experiments varied between 24° and 27°C.

When development was complete, the paper was removed from the jar and the forward boundary marked immediately with pencil. It was then dried in an upright position in a 70°C. oven. The dry paper was sprayed lightly four times with the following solutions in the order listed: hydrochloric acid, sodium nitrite, ammonium sulfamate, N-(1-naphthyl)-ethylene-diamine dihydrochloride. The red-purple spots marking the procaine or p-amino benzoic acid sites appear immediately after the final spraying.

The apparatus is employed as illustrated in Figure 13.

Results - Of the numerous standard solutions and samples chromatographed, the following are representative and provide results satisfying the objectives of this study:

1. Standard procaine hydrochloride solution, 0.01 ml of a 0.1% solution.

2. Standard p-amino benzoic acid solution, 0.01 ml of a 0.1% solution.

3. 1 plus 2. 1 was permitted to dry on the paper before the application of 2.

4. Commercial frostbite solution, 9 months old, 0.01 ml.

5. Laboratory frostbite solution, 0.01 ml. This is the same as 4, except that it was prepared in the laboratory and tested immediately after its preparation.

6. "New" commercial procaine solution for dental anesthesia diluted to contain 0.1% procaine hydrochloride.

7. "Old" commercial procaine solution for dental anesthesia diluted to contain 0.1% procaine hydrochloride. Unlike the preparation of 6, this solution was highly discolored.

The results are illustrated diagramatically in Figure 14. This is a composite representation from several experiments and variations in R_f values reflect the inadequacy of the type of paper used.

Throughout the diagram, procaine spots appear in the range of R_f 0.63 to 0.68, those of p-amino benzoic acid between R_f 0.85 and 0.88. Mixtures of procaine and p-amino benzoic acid make clear separation (point 3). The size and intensity of color of the spots are a fair indication of the amounts of material present, and it is seen in the comparison of the "new" and "old" commercial dental procaine solutions (points 6 and 7) that even where there has been great discoloration of the solution on aging, the breakdown of procaine with the consequent release of p-amino benzoic acid is only very slight.

The spots obtained with the frostbite solutions (points 4 and 5) substantiate the early suspicion that procaine combines with glucose. In addition to the procaine spots, we find spots at R_f 0.26 and 0.23. Theoretically, those substances which are more soluble in butanol with respect to their solubility in water should migrate most rapidly under the conditions described here. Thus, the acetate of p-amino benzoic acid with
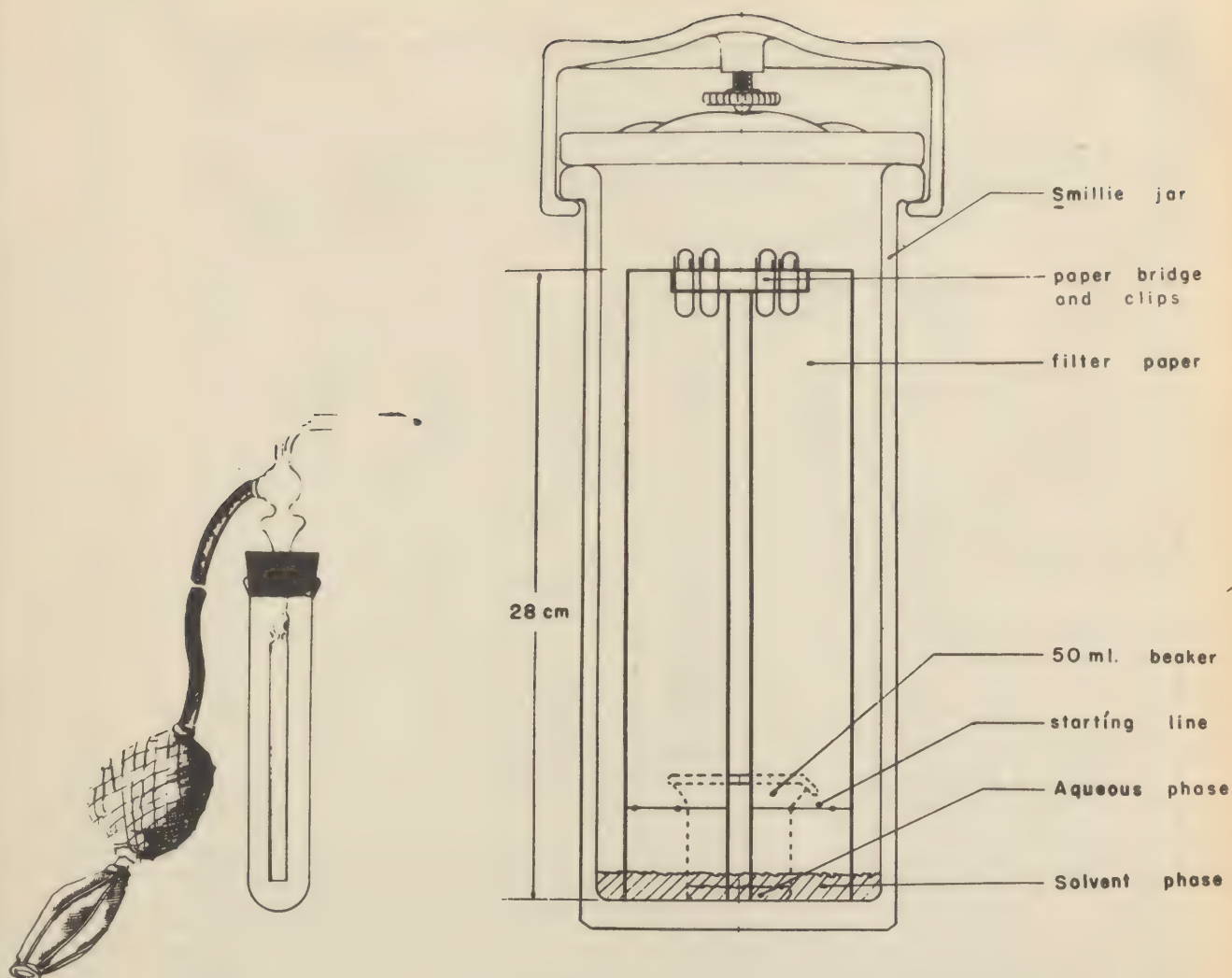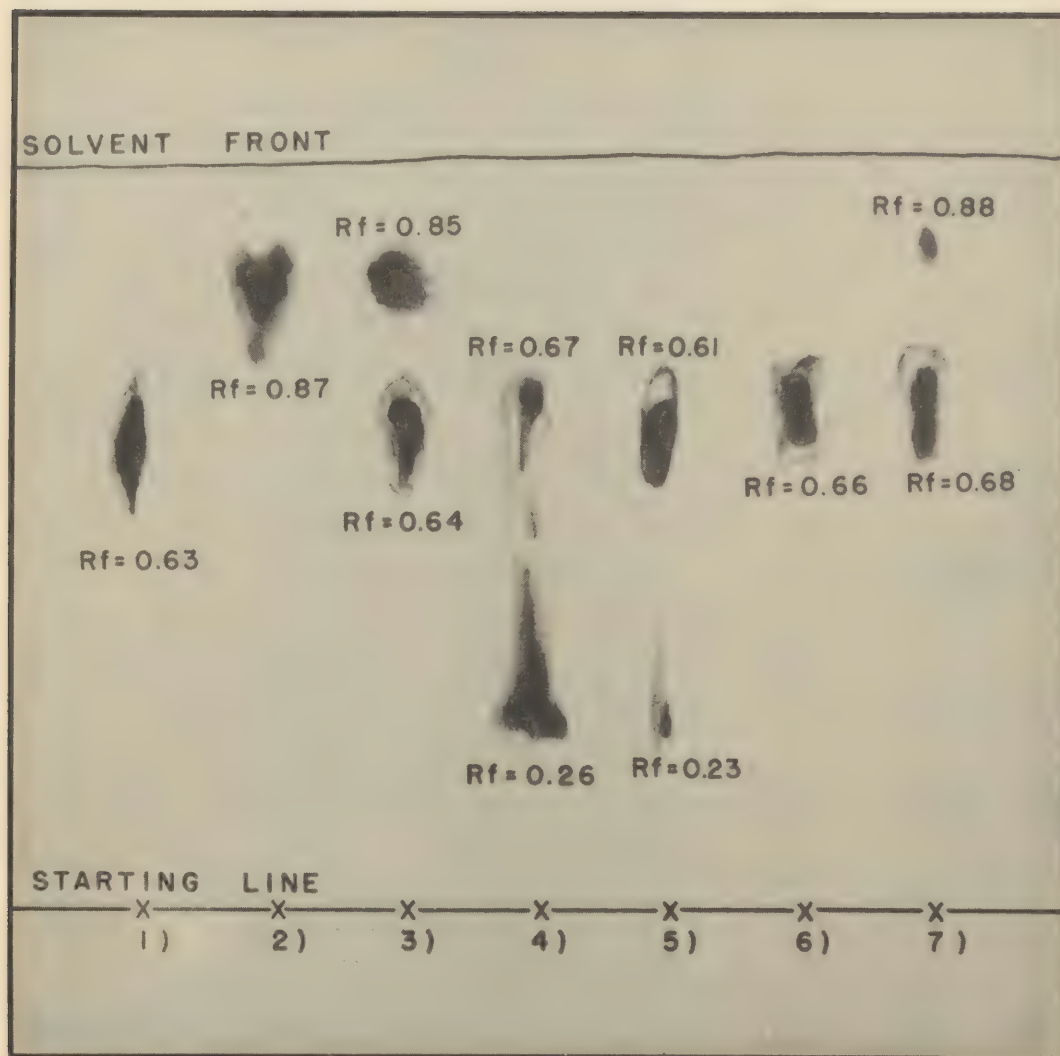
Figure 13. Apparatus For Paper Chromatography

Figure 14. Paper Chromatographs of Procaine Solutions

an R_f value of about 0.87 would be expected to be more soluble in butanol than pro-caine hydrochloride with its R_f value of about 0.65. It might also be expected that any combination of procaine with glucose would result in a compound of greater water solubility and less butanol solubility than procaine itself, and that such a com-pound would have a slower migration rate (i.e., lower R_f value). This is exactly what is found. It will be noted in the diagram that in the case of the 9-month-old frostbite solution (point 4) much more of the procaine is combined with glucose than exists in the free state. Even in the freshly prepared "laboratory frostbite solu-tion (point 5), the chromatograph indicates that a small amount of the procaine-glucoside is subject to acid hydrolysis, and it is very likely that the "trail" re-sults from the gradual hydrolysis of this compound during the development of the chromatograph, the hydrolysis being effected by the acetic acid of the solvent phase. Evidence supporting this view was gained later in experiments where neutral butanol replaced the acetic acid-butanol.

When the neutral butanol solvent was employed a chromatograph similar to that obtained above resulted. The migratory rate was but slightly retarded in the case of procaine, but the progress of the procaine glucoside (R_f = 0.11) and of p-amino benzoic acid (R_f = 0.48) was greatly delayed. The "trail" mentioned in the previous paragraph was absent.

When the ammonia-butanol solvent was used, the rate of procaine migration was greatly accelerated (R_f= 0.91) as compared with that obtained with the acetic acid-butanol mixture. Conversely, the p-amino benzoic acid moved hardly at all (R_f= 0.05). The procaine glucoside spot was missing where the frostbite solution had been devel-oped, suggesting that it had been hydrolyzed by the alkalinity of the solvent. This reversal in migration rates of procaine and p-amino benzoic acid as the solvent is changed from acid to alkaline is in keeping with expectations. The free procaine formed under alkaline conditions would be more butanol soluble than the procaine salt formed under acid conditions, and p-amino benzoic acid is more water soluble as the ammonium salt than as the acetate.

Since the colorimetric method is more convenient and suited to our purposes than the U.S.P. method, it was desired to determine its adaptability to frostbite solutions. It was soon discovered that, like the U.S.P. procedure, the colorimetric method does not measure the procaine of procaine glucoside. This is expected because procaine combines with glucose through the primary aromatic amino group, the group upon which the colorimetric procedure depends. It was also found, using both chem-ical procedures and the chromatographic technique, that procaine glucoside is readily hydrolyzed by mild acid or alkaline treatment. In this laboratory, frostbite solu-tions are now treated in the following manner before being diluted for analysis: 5 ml of frostbite solution is heated in a boiling water bath for five minutes with 2 ml of approximately 4 N hydrochloric acid. Numerous samples have been tested using all three methods, but the results obtained on a 9-month-old frostbite solution (Table XXII) will serve to illustrate the comparison:

Table XXII. Analyses of a 9-Month-Old Frostbite Solution by Three Methods

Method	Mg Procaine HCl per 100 ml
U.S.P.	24
Colorimetric without acid hydrolysis	43
Colorimetric with acid hydrolysis ..	102

The efficacy of acid hydrolysis is thus well demonstrated. That some of the procaine of the procaine glucoside is measured by the colorimetric procedure without acid hydrolysis is evident from the discrepancy in results found between that meas-urement and the results obtained using the U.S.P method. In the colorimetric method, 4 N hydrochloric acid is employed just before diazotization, and it is probable that some hydrolysis of procaine glucoside occurs at this time.

Discussion- While these studies were in progress, a publication was uncovered by Sannie and Vincent (55) who describe the isolation of a procaine glucoside from solutions containing both glucose and procaine. The hydrochloride of this compound melted at 130-131°C. and was very soluble in water, ethyl alcohol, and methyl alcohol. It was readily hydrolyzed by acid or alkali and even by simple warming in neutral solution, although in the last instance the hydrolysis was incomplete. Undoubtedly, this is the same compound encountered in our frostbite solutions. These authors question the physiological usefulness of procaine glucoside as the result of studies involving the anesthetic effect of both procaine and its glucoside on the oculo-palpebral reflex of the rabbit eye. They found the glucoside to have no anesthetic effect. They also mention (without giving references) failures by certain physicians to obtain anesthesia when glucose-procaine solutions are employed parenterally. It should be pointed out here, however, that too broad conclusions should not be drawn from experiments involving the topical anesthetic effects of procaine glucoside on the eye. Intravenous procaine glucoside could prove as effective as free procaine if enzyme mechanisms for hydrolysis should exist systematically. On the other hand, these studies raise a definite question as to the usefulness of procaine in frostbite solutions or other glucose-containing solutions, especially where they have been permitted to stand sufficiently long for a major part of the procaine to have combined with glucose.

Conclusions -

1. A paper partition chromatographic technique is described for the detection of procaine and its integrity in solutions commonly used in medical practice.

2. The investigation of frostbite solutions containing glucose (5%), alcohol (5%) and procaine hydrochloride (0.1%) revealed that a reaction occurs between glucose and procaine with the probable formation of procaine glucoside.

3. The U.S.P. and colorimetric methods fail to measure the procaine of procaine glucoside. The colorimetric method can be applied if the glucoside is first hydrolyzed with dilute hydrochloric acid.

4. A question is raised as to the efficacy of procaine in parenteral solutions containing glucose.

DEPARTMENT OF ENTOMOLOGY

During 1951 activities of this department were devoted mainly to studies on the role of mosquitoes in the epidemiology of Japanese B encephalitis, studies on resistance of lice to DDT, studies on the vectors of malaria on Formosa and of the vectors of scrub typhus in the Pescadores Islands. The number of arthropods identified during the year showed a sharp drop from the number made during 1950, due primarily to the markedly smaller mosquito population in the Tokyo area during 1951. A summary of arthropod identifications and other tests conducted by the department during 1951 is presented in Table I.

Table I. Summary of Activities

Adult Mosquito Identifications	68,677
Larval Mosquito Identifications	6,042
Other Arthropod Identifications	189
Rodent Identifications	269
Mosquito Blood Precipitin Tests	2,417
Mosquito Virus Isolation Tests	931*
Insecticide Tests	88
Total	78,613

* Of this number, 639 were tests conducted after 1 January 1951 on material collected during 1950.

Organization and activities of the department were expanded during the year to include an Ecological Subsection at Omiya and a Taxonomic Entomology Subsection at Kyoto. The latter was being organized late in the year and will be completely staffed early in 1952. Also being organized during the last month of the year was a field survey team for the study of rodents and rodent ectoparasites.

MOSQUITO SURVEYS IN RELATION TO JAPANESE B ENCEPHALITIS: Studies were continued in 1951 on the relation of mosquitoes to the epidemiology of Japanese B encephalitis. A total of 63,806 adult mosquitoes were taken in the course of these studies. Of this number, 12,563 mosquitoes were sealed in 308 tubes, quick frozen and preserved on dry ice for virus isolation tests. In addition, 164 lots containing 6,012 mosquitoes were turned over to the Department of Virus and Rickettsial Diseases for virus isolation tests. Twenty-one of these lots were tested in the fresh state for the presence of virus, and the remainder were frozen and tested 1-5 days later.

Mosquito collections during 1951 were made from adult resting stations, from light traps and bait traps and by human biting collection. Efforts were made to maintain collections throughout the breeding season on an unvarying basis, so that the observed results would closely reflect population changes.

Adult resting station collections were de-emphasized during the 1951 survey in Tokyo for reasons indicated in the 1950 Annual Report (1). During the season a total of 90 collections were made, yielding 12,058 mosquitoes. These collections were undertaken primarily for the purpose of obtaining mosquito material for virus isolation tests, and were not run on a regular weekly basis to obtain population data. Collections were made intermittently from 24 June until 23 September, mainly from horse stables, dairies, pig pens and bird observation blinds. The following species were taken in largest numbers: Culex pipiens pallens, Culex tritaeniorhynchus, Anopheles hyrcanus sinensis, and Aedes vexans nipponii.

Routine weekly light trap collections were begun on 1 May, utilizing four light traps, and were continued through the end of September. Each of these traps was operated three times weekly as follows: (1) inside a horse stable at Yoyogi, (2) outside

animal sheds at the Ueno Park Zoo, (3) inside the Tokyo Metropolitan Police stables, and (4) inside a horse stable at Edogawa during the earlier part of the season and then for the remainder of the season out-of-doors at the Shinhama (Niihama) bird refuge. A total of 17,582 mosquitoes were taken from 211 trap-nights of operation. The yield from light traps during 1951 was very markedly lower than catches in 1950. The average catch per trap night during 1951 was only 83 in contrast to an average of 751 trap night during 1950. This was not only true of light traps as a whole but also was evident in the individual light traps which were operated in the same location and in the same manner during the two years. The proportional breakdown of light trap catches during 1951 by species showed little variation from that obtained during the two previous years, with Culex tritaeniorhynchus being the predominant species. Population curves for Culex tritaniorhynchus derived from light traps during the years 1949-1951 are indicated in Figure 1. The peak week for the species during 1951 was the week beginning 29 July. It should be noted that light traps were less efficiently placed during 1949 and that the collection curve obtained during that year may not be exactly comparable with the two following years.

Light traps were operated intermittently early in the season in efforts to obtain early emerging Culex tritaeniorhynchus for virus isolation tests. From a total of 13 trap-nights of operations only 1 trap yielded any C. tritaeniorhynchus. This catch consisted of 5 females, 4 of which were engorged with blood, and was taken from a light trap operated at a dairy in Edogawa on 25 April 1951. This is the earliest date on which adults of this species have been taken in three years of studies, but it should be noted that in 1949 and 1950, mosquito collections did not commence until about mid May.

Five animal bait traps were operated during 1951, each for three nights per week. The locations and bait animals in these traps were as follows: (1) Yoyogi Stable - horse, (2) Yoyogi Stable - 20 chickens, (3) Ueno Park Zoo - horse, (4) Ueno Park - 20 ducks, and (5) Niihama (Shinhama) Bird Refuge - 5 egrets, 5 herons. (During April and May this trap was operated at Edogawa with a horse as bait). Bait trap operations extended from early April until the end of September. Trap-nights of collection totalled 315 with a yield of 12,631 mosquitoes. During the entire season, five normal 15 day old mice were placed in each bait trap to determine if trapped mosquitoes could transmit Japanese B encephalitis to these mice through the bite. The details and results of this project are discussed later in this report. A bait trap was operated at Edogawa with a horse as bait early in the season in efforts to obtain early emerging Culex tritaeniorhynchus. In nine nights of operation during April and early May, only one female C. tritaeniorhynchus was taken, this on the night of 2 May 1951.

A breakdown of bait trap catches from equine and avian baited traps is provided in Table II. It is of particular interest to note that the predominant species taken from avian baited traps was Culex tritaeniorhynchus. Prior to the inception of the trapping with avian bait in 1951, it was believed that Culex tritaeniorhynchus did not attack birds, since the species was rarely taken in adult resting station collections in chicken houses and because of the very small proportion of avian positives in the 1950 precipitin test series on blood smears from engorged C. tritaeniorhynchus (1). A further breakdown on results obtained with avian baited traps is provided in Table III, which lists catches from each of the avian baited traps. It will be noted that there is a considerable discrepancy between the catches from the various traps, particularly in the proportionate yields of Culex pipiens and Culex tritaeniorhynchus. It would be very hazardous, at present, to accredit these results purely to innate differences in the attractiveness of the different birds used as bait. While such differences may be responsible for the results obtained, it is well to bear in mind that the variation in catches may be due wholly or in part to the placement of the traps in different localities. The effects of locality on trap yields, where horses and cows were used as bait, was demonstrated during 1950 (1). For elucidation of this question, it will be necessary to operate two or more bait traps containing a given species of bird at two or more localities.
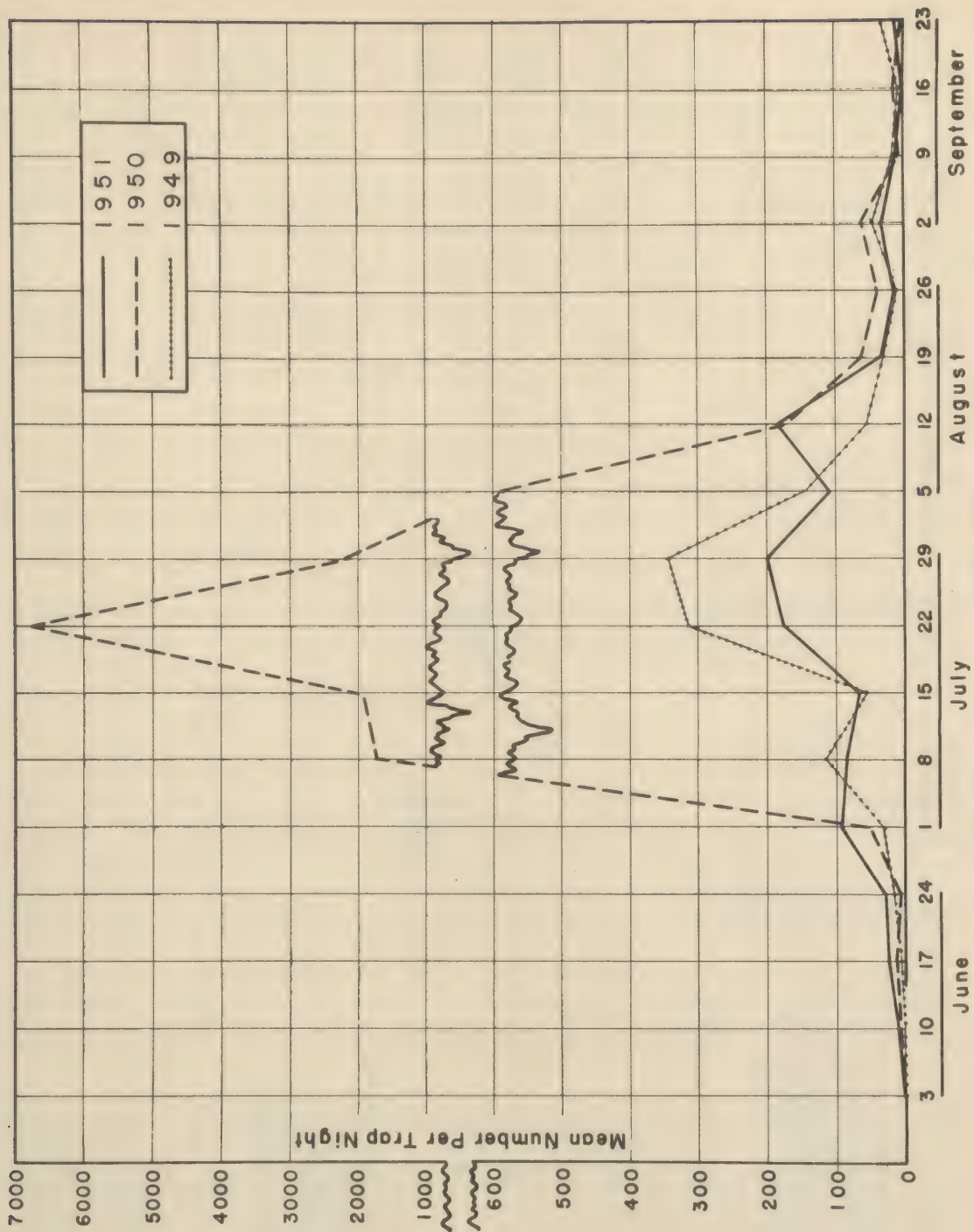
Figure 1. Light Trap Collection Curves – Culex tritaeniorhynchus: 1949-1951

Table II. Summary of Animal Bait Trap Collections

Species	Horse Bait		Avian Bait		Totals	
	No.	No. Per Trap-Night	No.	No. Per Trap-Night	No.	No. Per Trap-Night
Anopheles hyrcanus sinensis	516	4.9	12	0.1	528	2.1
Armigeres subalbatus	521	5.0	71	0.5	592	2.4
Aedes vexans nipponii	931	8.9	16	0.1	947	3.8
Culex pipiens pallens	63	0.6	2771	19.0	2834	11.3
Culex tritaeniorhynchus	3979	37.9	2912	20.0	6891	27.5
Culex bitaeniorhynchus	27	0.3	8	0.1	35	0.1
Misc. spp.	36	0.3	13	0.1	49	0.2
Totals	6,073	57.8	5803	39.8	11,876	47.3
No. trap nights	105		146		251	

Table III. Collection Results from Bird Baited Traps

Species	Yoyogi Stable (Chickens)		Ueno Park (Ducks)		Nihama (Egrets and Herons)		Totals	
	No.	No. Per Trap-Night	No.	No. Per Trap-Night	No.	No. Per Trap-Night	No.	No. Per Trap-Night
Anopheles hyrcanus sinensis	1	0.1	1	0.1	10	0.3	12	0.1
Armigeres subalbatus	70	1.1	1	0.1	0	0	71	0.5
Aedes vexans nipponii	5	0.1	11	0.3	0	0	16	0.1
Culex pipiens pallens	594	9.0	1846	44.0	331	8.7	2771	19.0
Culex tritaeniorhynchus	605	9.2	314	7.5	1993	52.5	2912	20.0
Culex bitaeniorhynchus	5	0.1	1	0.1	2	0.1	8	0.1
Misc. spp.	8	0.1	4	0.1	1	0.1	13	0.1
Totals	1288	19.5	2178	51.9	2337	61.5	5803	39.8
No. trap nights	66		42		38		146	

Seasonal trends in mosquito populations were measured in Tokyo during 1951 by two horse baited traps, one placed in the Yoyogi stable and the other in the Ueno Park Zoo. As was found in the operation of light traps, catches from bait traps were markedly lower than those obtained in 1950. During the 1950 season the mean number of mosquitoes taken per trap night from horse baited traps was 563, while in 1951 this number was reduced to 58, the biggest reduction in 1951 occurring in the Culex tritaeniorhynchus catches. Seasonal trends in Anopheles hyrcanus sinensis, Armigeres subalbatus and Aedes vexans nipponii, as measured by horse baited traps, are indicated in Figure 2. The number of Culex pipiens taken in these traps are so small that it is impossible to prepare a population curve from this material. The population curve for Culex tritaeniorhynchus obtained from horse baited traps is presented in Figure 3 together with the curves obtained by this method during 1949 and 1950. The greatly reduced level of the curve during 1951 is quite striking and it will be noted that the characteristic sharp mid-summer peak for this species was not evident during 1951. The week beginning 22 July yielded the largest catches of Culex tritaeniorhynchus as can be seen from Figure 3, but the level attained was much too low to produce a sharp peak.

Early in July, one bait trap was operated at Yoyogi stable with a tank of liquid CO_2 adjusted so that gas slowly evolved to serve as an attractant to mosquitoes, in an effort to obtain unengorged Culex tritaeniorhynchus for Japanese B encephalitis virus transmission studies. After eight nights of operation this trapping procedure was abandoned since only 104 mosquitoes were taken of which 70 were Culex tritaeniorhynchus. A double screened cage was then placed inside the bait trap and a small pig placed in this cage to serve as bait. During 43 nights of operation this trap yielded 670 mosquitoes, 518 of which were Culex tritaeniorhynchus. A small proportion of the mosquitoes taken from this pig baited trap were engorged, indicating either that the double screening enclosing the pig was not entirely effective or that some engorged mosquitoes were entering the bait trap. In connection with this latter possibility, see the section in this report devoted to precipitin tests on mosquitoes taken in bait traps.

During the mosquito breeding season of 1951, human biting collection of adult mosquitoes was given greater emphasis than in the previous year. This was done by doubling the scale of the 1950 program and by broadening the scope to include rural as well as urban collection areas. Two hundred biology students from eight high schools and middle schools in Tokyo or nearby rural areas cooperated in this program by undertaking two hours of biting collection each week. The organization and procedures in this program were essentially the same as those used in 1950 (1). Each of the eight schools provided 25 students for the biting collection program, and it was found, as in 1950, that directions for collection were faithfully carried out. The schools with their location and collection schedules are listed in Table IV. The level of collections remained fairly constant throughout the season, averaging 375 man-collection-hours per week. During the season, a total of 5,628 man-hours of collection were made, with a total yield of 12,726 mosquitoes. Human biting surveys were begun on 17 June and were terminated on 29 September.

A breakdown of collections from the four urban schools on an hourly basis is presented in Table V, while a similar breakdown for the four rural schools is given in Table VI. As was found in 1950 (1), no clear-cut biting trends are indicated by any of the species involved. This is somewhat surprising since it has been demonstrated by numerous studies on mosquitoes in several areas of the world (2, 3, 4, 5, 6, 7) that adult mosquitoes generally tend to show distinct biting trends with reference to time of day. One possible explanation for the failure to demonstrate such hourly biting patterns may be the size of the catches, which are very small considering the large number of hours devoted to biting collections. With the exception of Culex pipiens it is questionable whether the catches are large enough to even attempt looking for hourly biting patterns. It is also possible that if biting collections were made over a longer period of the day, biting trends would become apparent. However, instances in which the mosquito fauna of an area do not show such biting patterns are not unknown (8), (9).
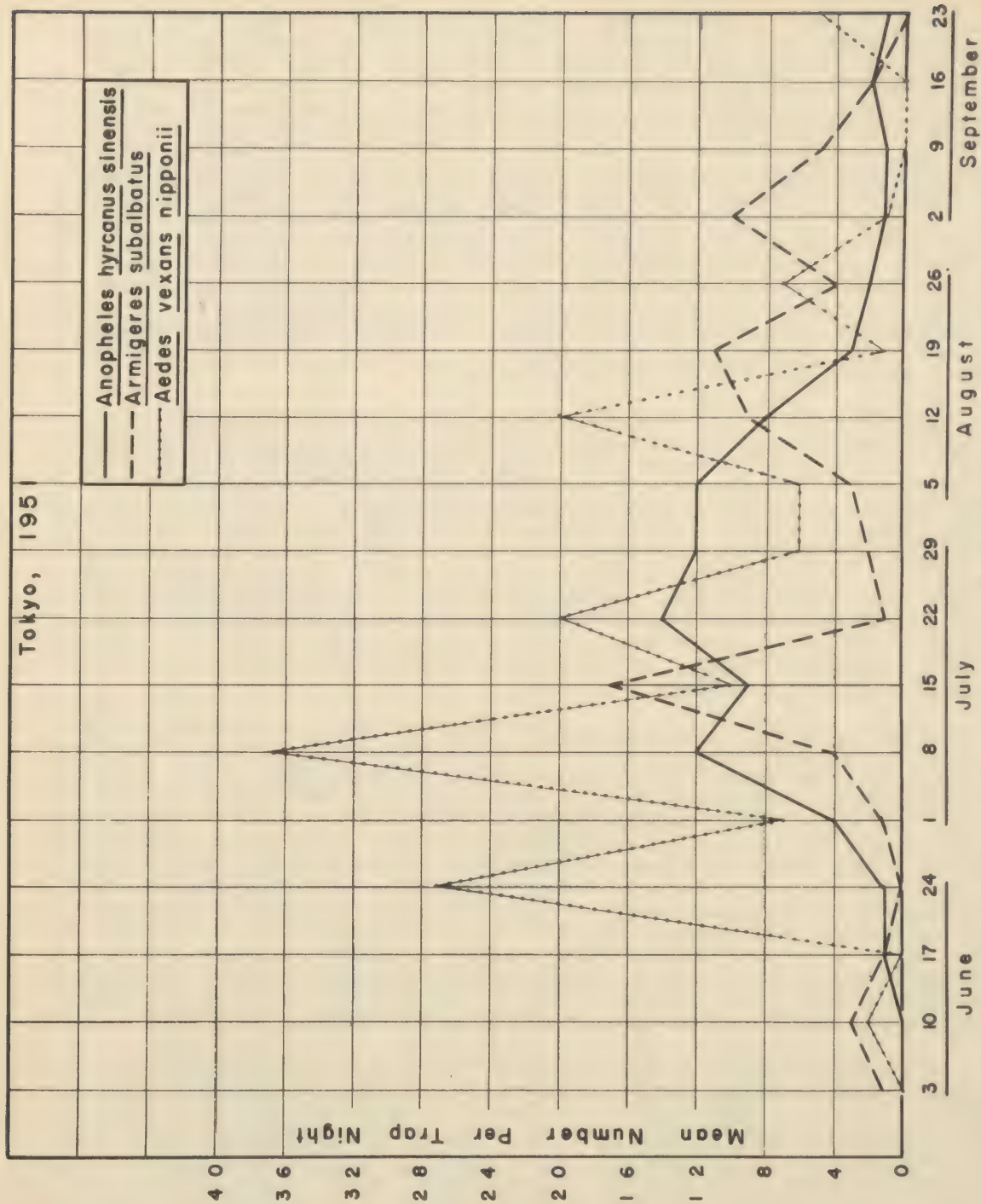
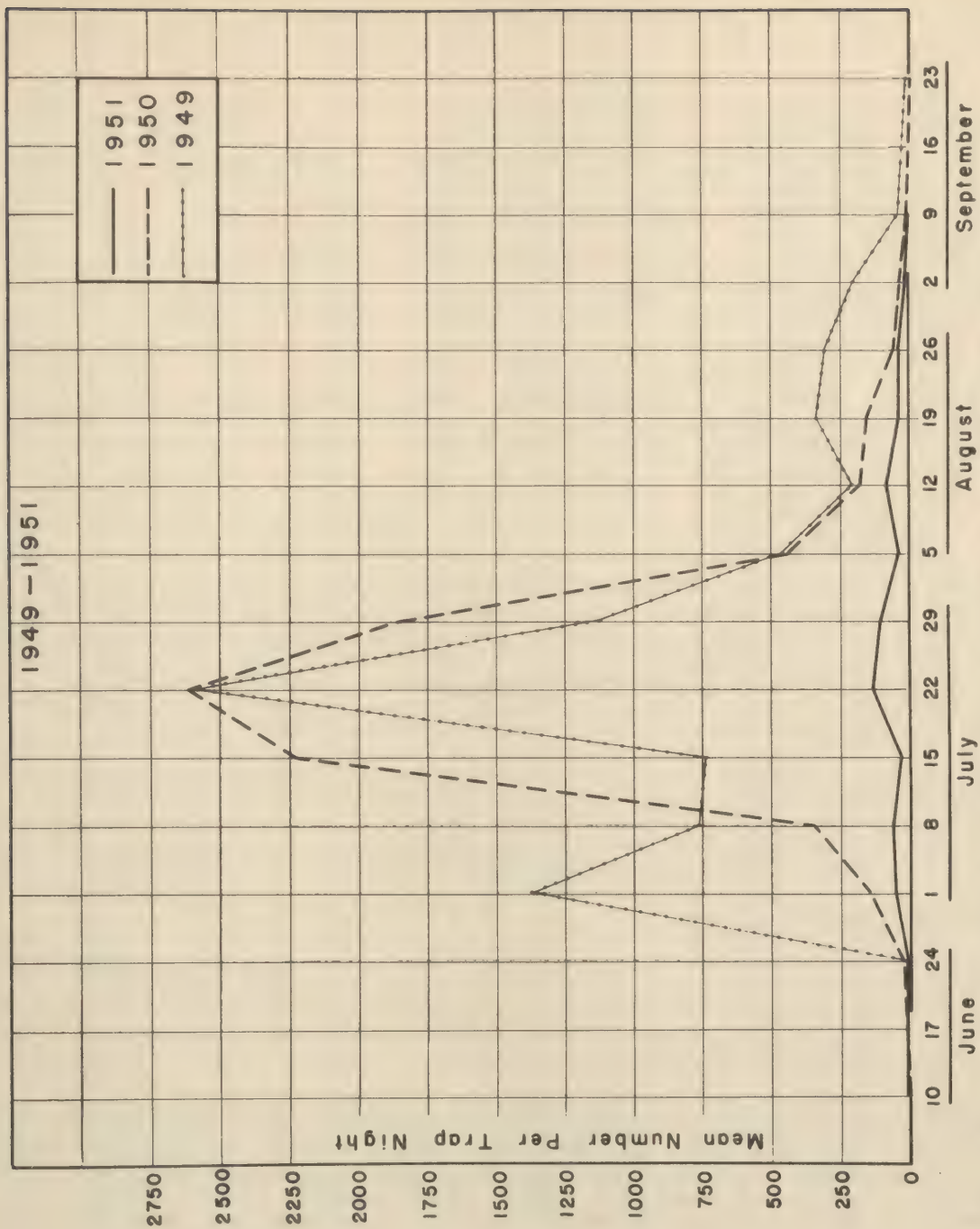Figure 2. Population Curves of Three Mosquito Species Based on Horse Baited Traps

Figure 3. *Culex tritaeniorhynchus* Population Curves Based on Horse Baited Traps, Tokyo

Table IV. Schools Scheduled in Human Biting Program

Name of School	Location	Rural or Urban	Schedule			Students
Mejiro H.S.	Shinjuku-Ku Tokyo	Urban	Mon. - Tues.	7-8	PM	Girls
Tokyo H.S.	Nakano-Ku Tokyo	Urban	Tues. - Wed.	8-9	PM	Boys
Ryogoku H.S.	Sumida-Ku Tokyo	Urban	Wed. - Thurs.	9-10	PM	Boys & Girls
Meguro M.S.	Meguro-Ku Tokyo	Urban	Thurs. - Fri.	10-11	PM	Boys & Girls
Yatsuka M.S.	Yatsuka, Saitama Pref.	Rural	Mon. - Tues.	7-8	PM	Boys & Girls
Jindai M.S.	Jindai, Kitatama-Gun	Rural	Tues. - Wed.	8-9	PM	Boys & Girls
Tachibana H.S.	Mizonokuchi, Kanagawa Pref.	Rural	Wed. - Thurs.	9-10	PM	Boys & Girls
Yamato M.S.	Yamato, Saitama Pref.	Rural	Thurs. - Fri.	10-11	PM	Boys & Girls

During the 1951 biting collection program, the mean number of mosquitoes taken per man hr. was 2.2, while in 1950 only 1.9 mosquitoes were taken per man hour. The increase in 1951 was due mainly to an increased catch of Culex pipiens, although the catch of Culex tritaeniorhynchus decreased markedly below that of 1950. Illustrating the extremely low yield from Culex tritaeniorhynchus is the population curve for this species during 1951 given in Figure 4, together with population curves obtained in 1949 and 1950. The curve shown in Figure 4 for 1949 is based on data obtained by Petrakis (10) in Yokohama, since we have no data for that year from Tokyo. The 1951 curve in Figure 4 illustrates the population trend shown by collections made in urban Tokyo in the same manner as the curve for 1950. While the peak of biting intensity for Culex tritaeniorhynchus occurred during the week of 22 July in urban Tokyo, the peak week in rural areas near Tokyo was the week of 8 July. However, the level of human biting rates by Culex tritaeniorhynchus during 1951 in both urban and rural areas was so low and biting activity fluctuated so widely that it is questionable if comparisons can properly be made.

During 1951, an attempt was made to correlate human biting rates of Culex tritaeniorhynchus with case rates of Japanese B encephalitis in the various kus (precincts of the city of Tokyo.) This analysis is discussed below in detail in the section of this report devoted to the relation of mosquito populations to the Japanese B encephalitis epidemic of 1951 in Tokyo.

RELATION OF WEATHER TO MOSQUITO POPULATIONS AND TO EPIDEMICS OF JAPANESE B ENCEPHALITIS: In our 1949 and 1950 reports (11, 1) attempts were made to interpret seasonal trends in mosquito populations in terms of weather factors, but without success. It would appear evident that weather should play an important role in determining the development of mosquito populations and of mosquito-borne diseases. There has been frequent reference in the literature to the association of large epidemics of Japanese B encephalitis with hot, dry summers (12, 13, 14), and much emphasis has been given to Mitamura's report (15), that the virus of Japanese B encephalitis will multiply most readily in the mosquito at the temperature range of 27°C. to 31°C. (80.6°F. to 87.8°F.). An examination of weather data during the 1949 and 1950 seasons failed to establish any clear-cut relationship between temperature or other weather factors and either mosquito population trends or epidemic trends.

Table V. Mosquito Collection Rates in Human Biting Survey, Urban Areas (Tokyo) - 1951

Mosquito Species	7-8 PM		8-9 PM		9-10 PM		10-11 PM		Totals	
	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.
Anopheles hyrcanus sinensis	16	.02	4	.01	11	.02	2	.01	33	.01
Armigeres subalbatus	8	.01	4	.01	7	.01	3	.01	22	.01
Aedes albopictus	25	.04	17	.02	3	.01	16	.02	61	.02
Aedes togoi	0	0	8	.01	1	.01	2	.01	11	.01
Aedes vexans nipponii	41	.06	14	.02	8	.01	25	.04	88	.03
Culex tritaeniorhynchus	98	.14	33	.05	41	.06	29	.04	201	.07
Culex pipiens pallens	1035	1.48	915	1.26	2371	3.35	776	1.15	5097	1.82
Culex bitaeniorhynchus	9	.01	12	.02	4	.01	6	.01	31	.01
Misc. spp.	2	.01	0	0	0	0	2	.01	4	.01
Total	1234	1.77	1007	1.39	2446	3.45	861	1.28	5548	1.99

Table VI. Mosquito Collection Rates in Human Biting Survey, Rural Areas (Near Tokyo) - 1951

| Mosquito Species	7-8 PM		8-9 PM		9-10 PM		10-11 PM		Totals	
	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.	No. Coll.	No./Hr.
Anopheles hyrcanus sinensis	70	.10	64	.09	94	.13	74	.11	302	.11
Armigeres subalbatus	149	.20	183	.26	22	.03	77	.11	431	.15
Aedes albopictus	68	.09	45	.06	3	.01	5	.01	121	.04
Aedes togoi	4	.01	0	0	0	0	0	0	4	.01
Aedes vexans nipponii	9	.01	21	.03	11	.02	20	.03	61	.02
Culex tritaeniorhynchus	52	.07	100	.14	80	.11	71	.10	303	.11
Culex pipiens pallens	1689	2.30	1121	1.61	2045	2.90	913	1.32	5768	2.04
Culex bitaeniorhynchus	14	.02	101	.14	24	.03	24	.09	163	.06
Misc. spp.	0	0	18	.02	1	.01	6	.01	25	.01
Total	2055	2.80	1653	2.38	2280	3.24	1190	1.72	7178	2.55
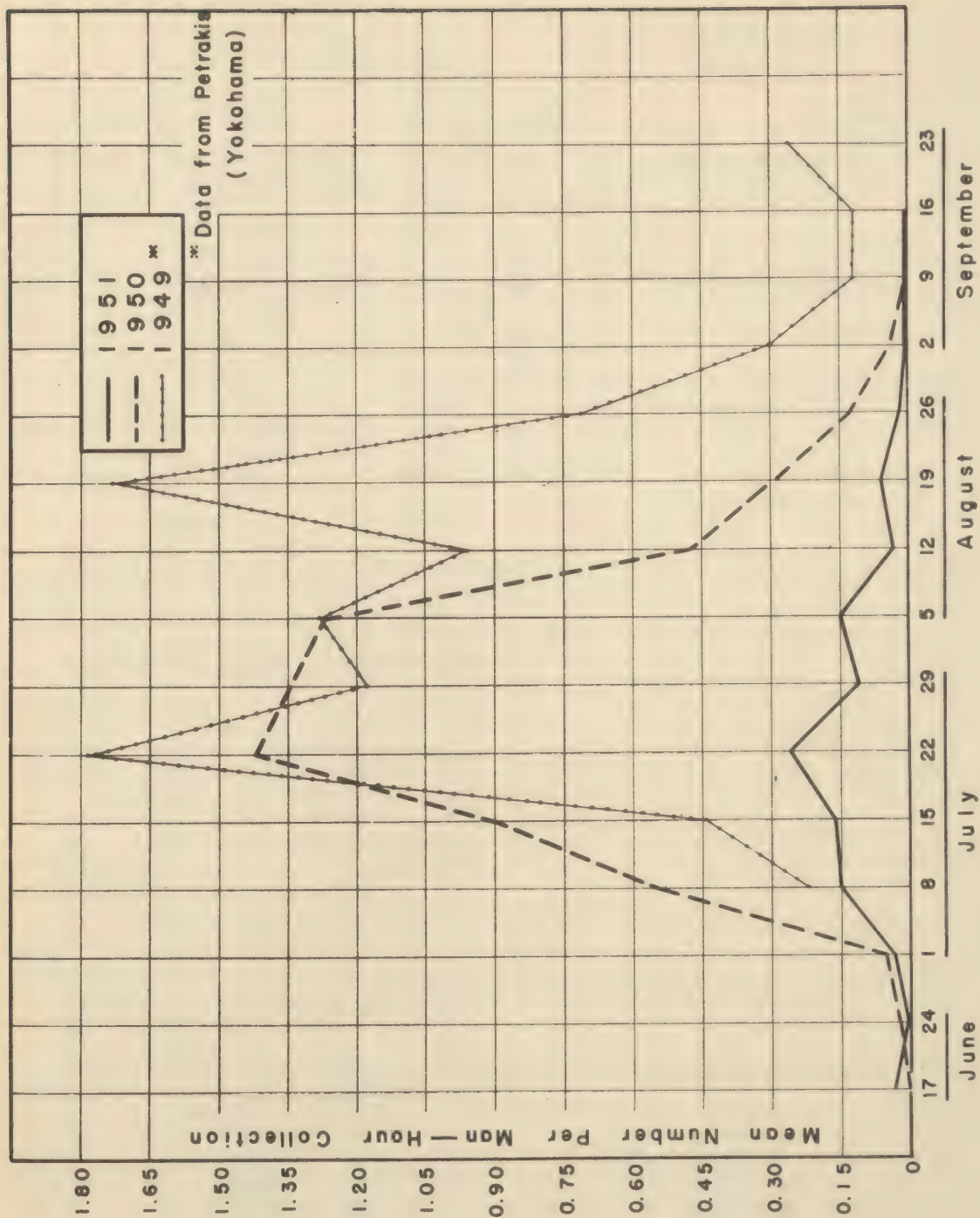
Figure 4. Human Biting Curves, Culex tritaeniorhynchus: 1949-1951

Comparison Between 1950, 1951 and Ten Year Averages - Weekly curves of mean temperature, mean saturation deficiency, total precipitation, mean relative humidity and total hours of sunshine for 1950, 1951 and for a ten year mean of the years 1930-1939 inclusive have been prepared in Figures 5-9. All data was obtained from the Central Meteorological Observatory in Tokyo. During 1950, 1304 cases of Japanese B encephalitis were reported from Tokyo, while 174 cases were reported during 1951. For a comparison of the population size of Culex tritaeniorhynchus in Tokyo during 1950 and 1951 see Figures 1, 3, 4. In Figure 5 it will be seen that the mean temperature curve for 1950 ran above the 10 year average during June and the first three weeks of July, a period in which Culex tritaeniorhynchus made a rapid growth in population size. The 1950 epidemic began during the fifth week of July and reached its peak in the second week of August. In 1951, the mean temperatures ran considerably below the ten year average during June and the first three weeks of July. Examination of Figure 6 for mean saturation deficiency indicates an even greater disparity between 1950 and 1951. While the mean saturation deficiency for 1951 was higher in June, during July the curve for 1950 exceeded the 10 year average by a considerable margin, and the curve for 1951 fell below the 10 year average to a comparable extent. In Figure 7 it will be seen that total weekly precipitation was quite variable during 1950, with heavy precipitation occurring during June and again in late July, while little precipitation occurred during 1951 until the third week of August. The mean relative humidity curves in Figure 8 are interesting because they show a generalized reversal of the saturation deficiency curves shown in Figure 6, while the curves for total hours of sunshine in Figure 9 generally follow the same trends exhibited by the saturation deficiency curves in Figure 6. In summary then, the difference noted between 1950, an epidemic year with a large Culex tritaeniorhynchus population, and 1951 a non-epidemic year with a low C. tritaeniorhynchus population are as follows:

temperature -	high during June and July of 1950
	low during June and July of 1951
saturation deficiency-	low during June and high during July of 1950
	variable during June and low during July of 1951
precipitation -	high during June, late July and early August of 1950
	low except in latter part of August of 1951
relative humidity -	high in June and August, low in July of 1950
	high except in late July and early August of 1951
hours of sunshine -	low in June and high in July of 1950
	variable during June, low during July of 1951

Comparison Between Five Epidemic Years, Five Non-epidemic Years and Ten Year Averages - Comparisons for limited periods, such as have been made above for 1950 and 1951, while of interest, are limited in value since it is difficult to evaluate the importance of any variables found. For this reason we have selected five epidemic years (1924, 1935, 1939, 1948, 1950) in Tokyo and five non-epidemic years (1931, 1932, 1934, 1938, 1951) in Tokyo and made comparisons with the 10 year averages of the various weather factors, based again on data from Tokyo. During each of the epidemic years listed above, there were over 1,000 cases of Japanese B encephalitis in Tokyo, while less than 200 cases occurred in Tokyo during each of the non-epidemic years. A similar approach has been used by LaCasse and Yamaguti (16) but unfortunately they compared weather data from Kyoto, Japan with the incidence of the disease in Japan as a whole.

In Figures 10-14 we have prepared a series of comparisons between the five epidemic years, the five non-epidemic years and the ten year mean with respect to weekly mean temperature, weekly mean saturation dificiency, weekly mean total precipitation, weekly mean relative humidity and weekly mean total hours of sunshine. Examination of Figure 10 for mean weekly temperatures reveals little deviation during either the epidemic or the non-epidemic years from the ten year mean, and little difference between the curves for epidemic and non-epidemic years. In Figure 11 it will be found that the mean saturation deficiency during the epidemic years ran higher during July than the ten year mean, and considerably higher than the level of the five non-epidemic years. This trend was reversed during August however, with the non-epidemic years showing higher mean saturation deficiencies than the epidemic years. Examination of Figure 12 for precipitation
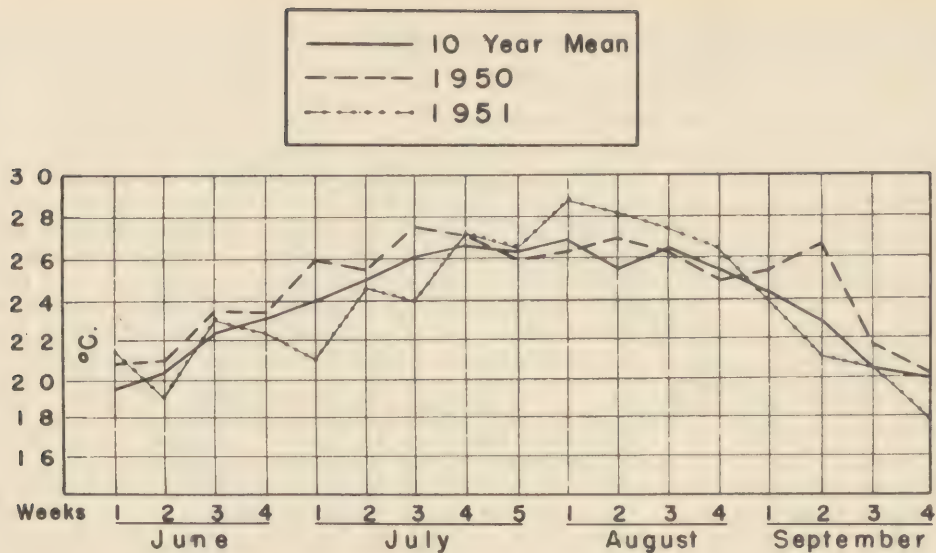
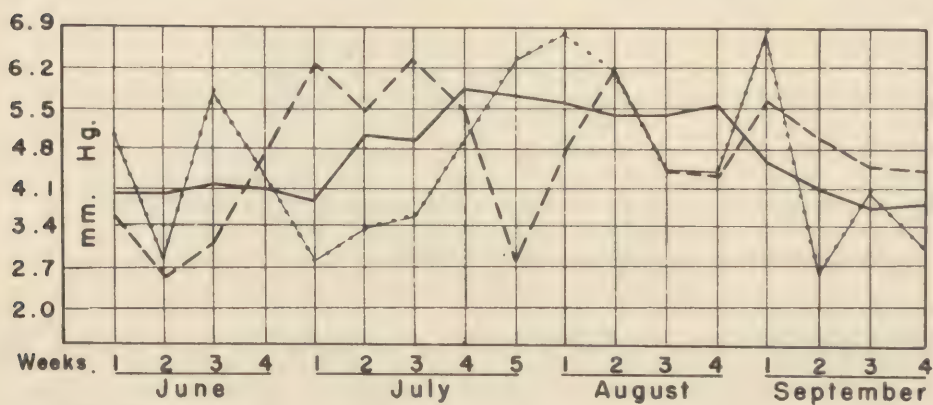Figure 5. Temperature-1950, 1951, and 10 Year Mean

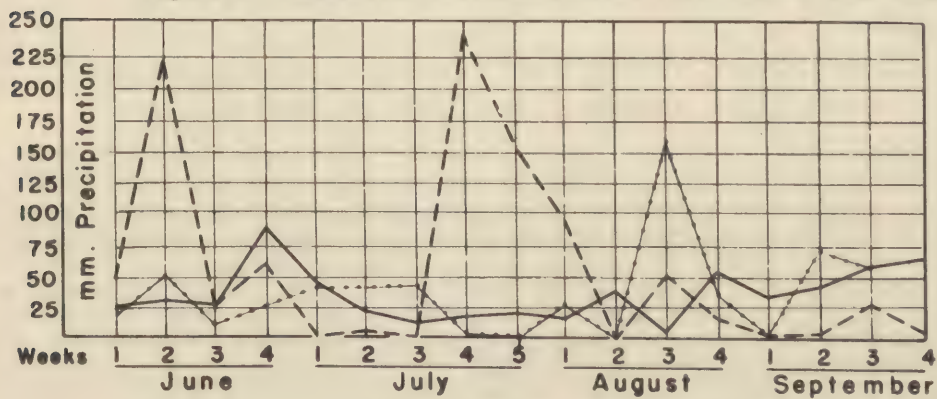Figure 6. Saturation Deficiency-1950, 1951, and 10 Year Mean

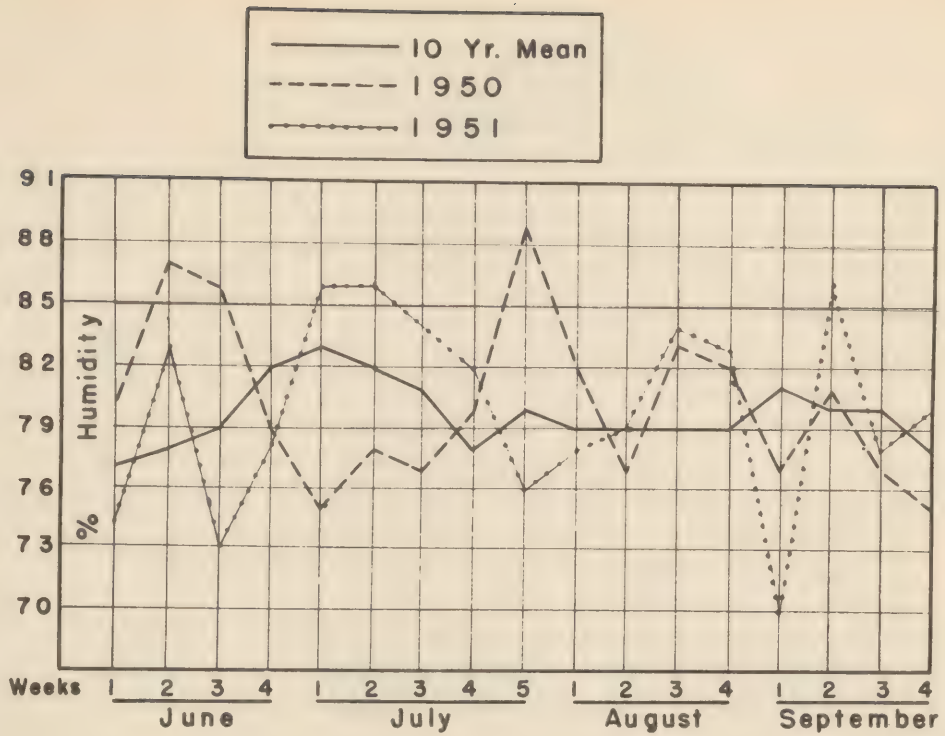Figure 7. Precipitation-1950, 1951, and 10 Year Mean

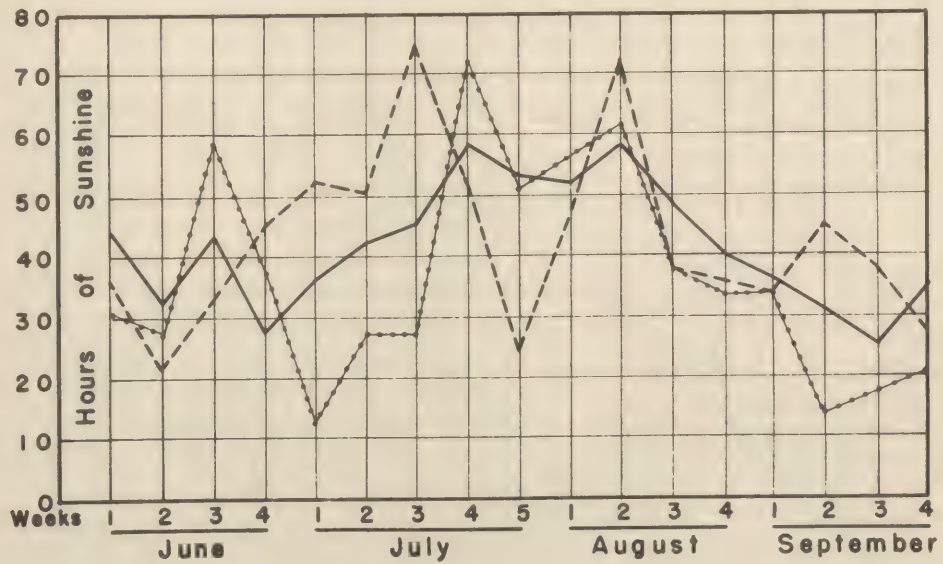Figure 8. Relative Humidity - 1950, 1951 and 10 Year Mean

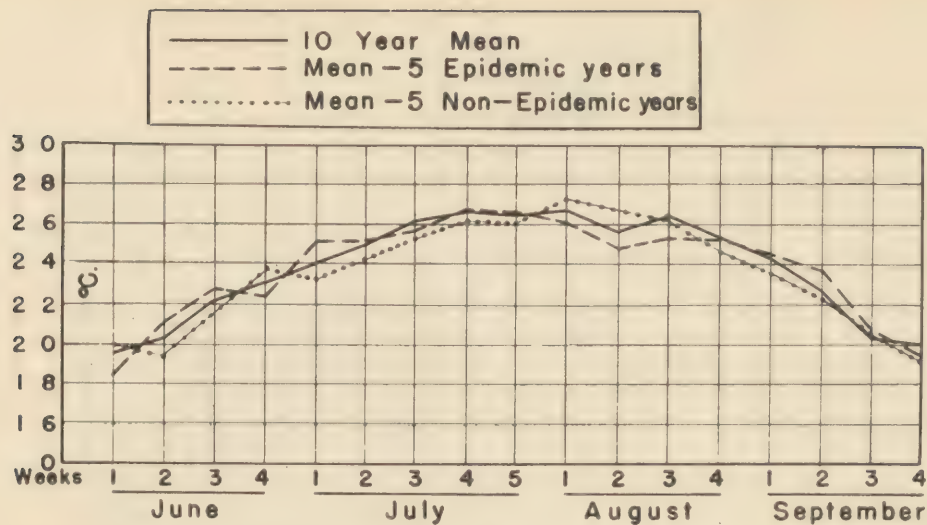Figure 9. Hours of Sunshine - 1950, 1951 and 10 Year Mean

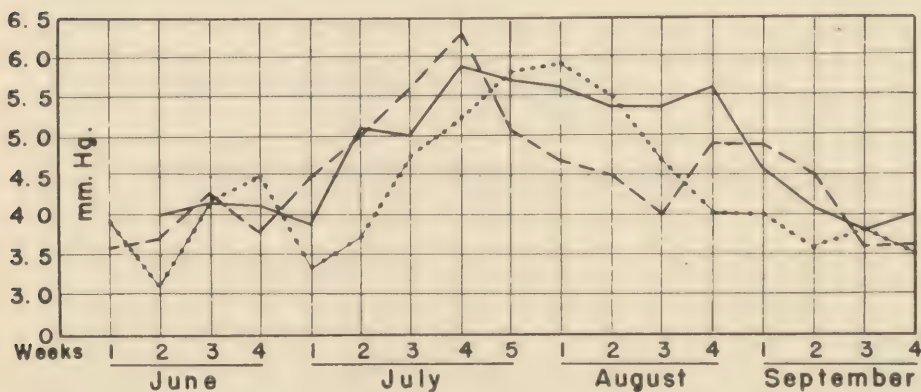Figure 10. Temperature-Epidemic and Non-Epidemic Years

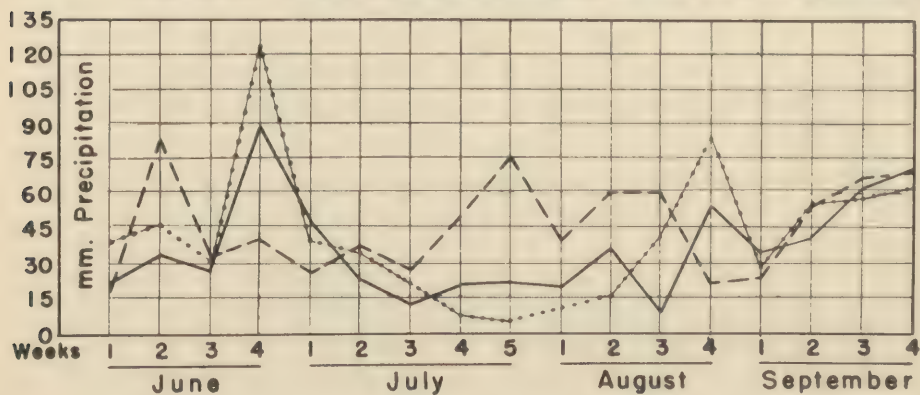Figure 11. Saturation Deficiency-Epidemic and Non-Epidemic Years

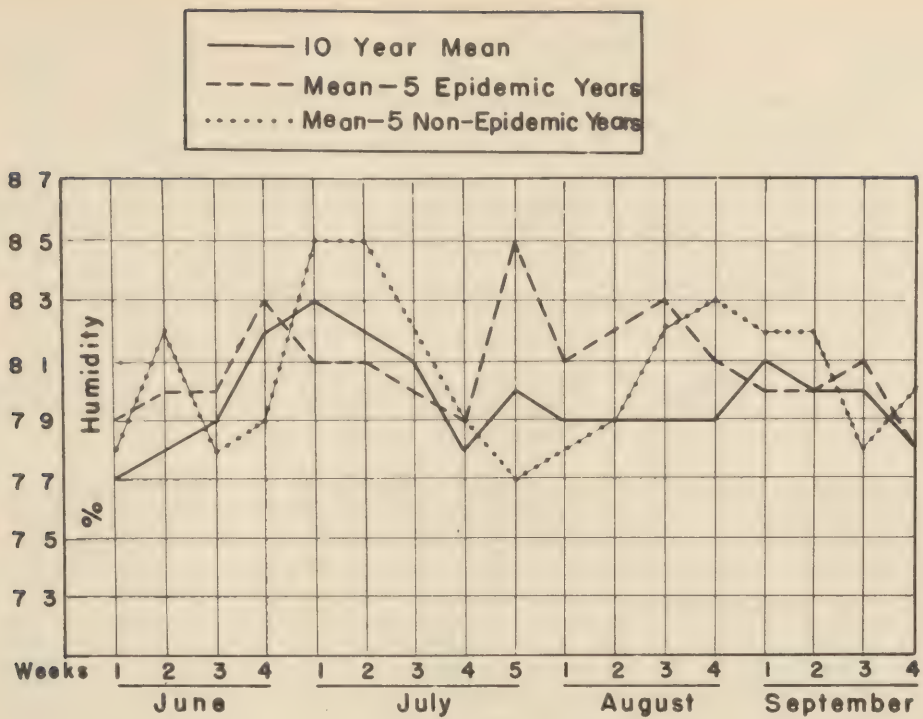Figure 12. Precipitation-Epidemic and Non-Epidemic Years

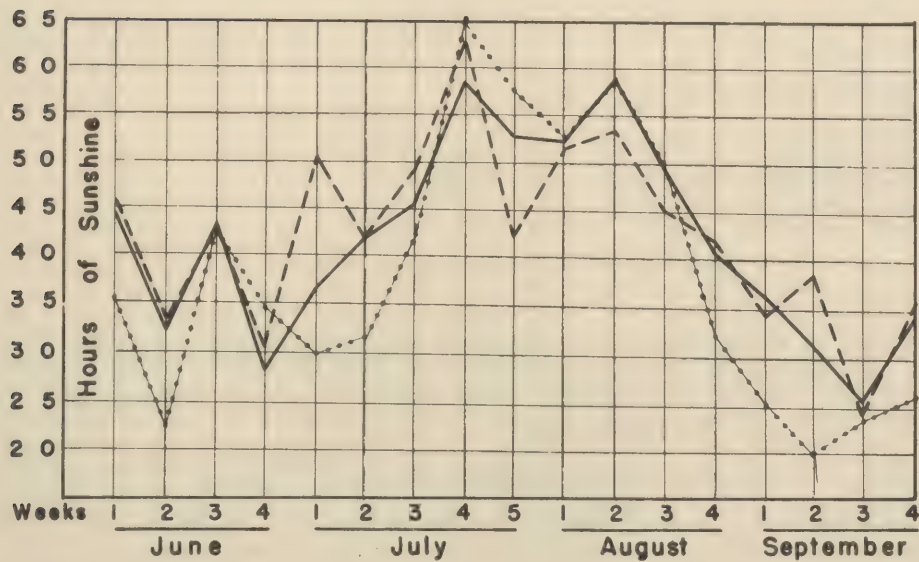Figure 13. Relative Humidity - Epidemic and Non-Epidemic Years

Figure 14. Hours of Sunshine - Epidemic and Non-Epidemic Years

shows a variable situation, with periods of high rainfall dispersed throughout the summer in both the epidemic years and in the non-epidemic years. Of possible interest, is the relatively high level of rainfall which occurred during the latter half of July and much of August in the five epidemic years, in contrast to the much lower levels during the same period of the non-epidemic years. The relative humidity curves in Figure 13 generally show a reversal of the saturation deficiency curves shown in Figure 11. Comparison of the curves on mean total hours of sunshine in Figure 14 reveal differences between the epidemic and non-epidemic years during July and early August, but nothing to indicate any trend of apparent significance. Whether or not any of the differences which are found in this series of graphs are of any significance is difficult to determine, but it is apparent that examination of Figures 10 and 12 dispute the frequent statement that epidemics of Japanese B encephalitis tend to occur during years in which the summer is hot and dry. We find ourselves in general agreement with LaCasse and Yamaguti (16) who concluded their analysis of weather data in relation to Japanese B encephalitis with, "In conclusion we can state that we are finally convinced that these environmental factors are probably quite favorable in all seasons and we must look elsewhere for possible clues ----".

RELATION OF MOSQUITO POPULATIONS TO JAPANESE B ENCEPHALITIS IN TOKYO IN 1951: During 1951 there were only 174 cases of Japanese B encephalitis reported in Tokyo. This was only 13% of the number reported during 1950. The most striking feature of the mosquito population studies conducted in Tokyo during 1951 was the greatly reduced yield of Culex tritaeniorhynchus, in comparison with catches of the previous year. This was evident by all methods of collection and is well illustrated in Figures 1, 3, and 4. The reduction in mosquito catches while most marked in the case of Culex tritaeniorhynchus applied generally to almost all species as shown in Table VII. The figures given in this table represent the mean number collected per trap night from light traps and from animal bait traps and the mean number collected per man-hour in human biting collections. Because of the smaller mosquito population in 1951, it has been more difficult to compare seasonal variations in mosquito populations with the 1951 epidemic of Japanese B encephalitis than was the case in 1950, and again it has been necessary to construct mosquito population curves on diversified bases.

Table VII. Mosquito Collection Rates During Peak Weeks, 1949-1951, Tokyo

Species	Light Traps			Bait Traps			Human Biting		
	1949	1950	1951	1949	1950	1951	1949*	1950	1951
Aedes albopictus	-	-	-	-	-	-	.49	.24	.06
Anopheles hyrcanus sinensis	4	76	4	108	63	14	.29	.08	.04
Armigeres subalbatus	-	-	-	4	48	17	.09	.29	.03
Aedes vexans nipponii	33	72	115	229	84	36	.06	.07	.13
Culex tritaeniorhynchus	173	6745	201	2583	2621	131	1.7	1.4	.26
Culex pipiens pallens	11	16	62	9	2	1.7	3.1	2.6	3.7

* Based on data from Petrakis, Yokohama

An examination of the variations shown in the Anopheles hyrcanus sinensis population curves in relation to the incidence curve of Japanese B encephalitis in Tokyo during 1951 shows curves with two or three peaks which drop off markedly four or five weeks prior to the peak of the epidemic (Figure 15). The yield of this species was so low in the human biting collection program, that it was impossible to construct a population curve. In Figure 16 the Culex pipiens pallens population curves prepared from human biting collections and from animal bait traps have been compared with the incidence of Japanese B encephalitis in Tokyo. While horse baited traps have been used as a basis of comparison for Anopheles hyrcanus sinensis and Culex tritaeniorhynchus, catches of Culex pipiens have been so low in horse baited traps, that it
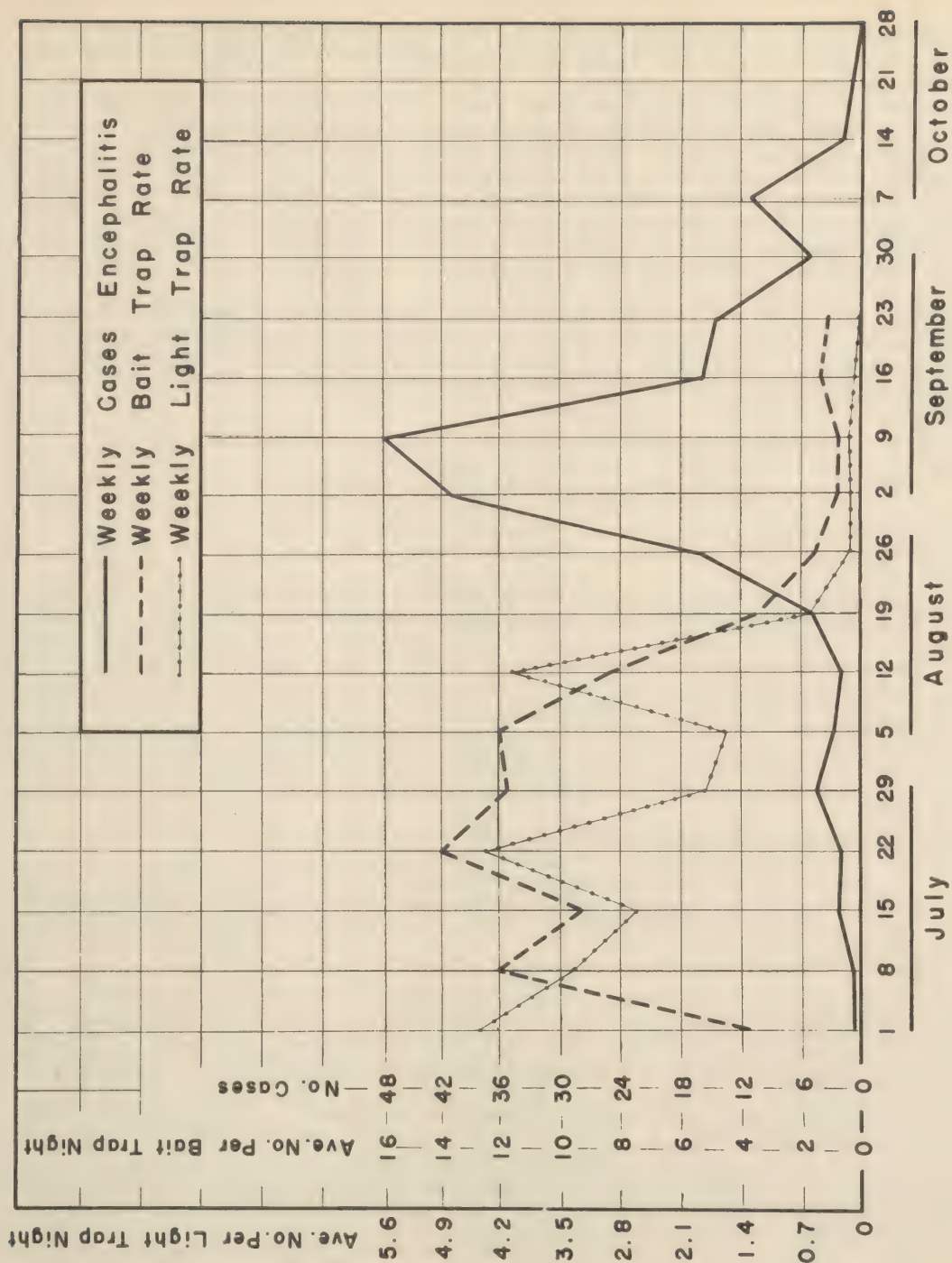
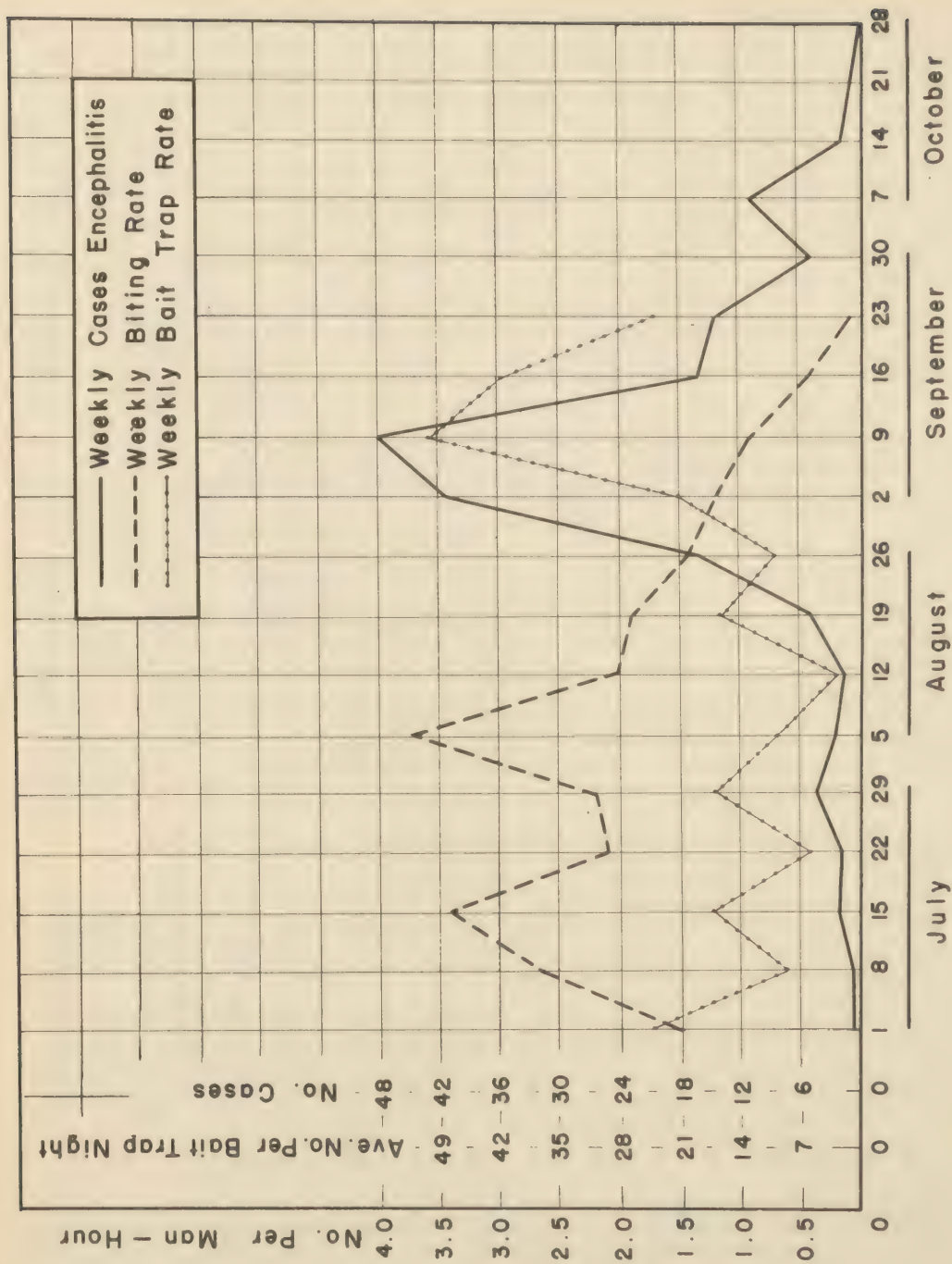Figure 15. Relation of A. hyrcanus sinensis to Tokyo JBE Epidemic of 1951

Figure 16. Relation of Culex pipiens pallens to Tokyo JBE Epidemic of 1951

has been necessary to construct a curve in this instance from bird baited traps. The two curves for this species differ so greatly, that little correlation with the incidence curve can be noted. Comparison of the incidence curve of Japanese B encephalitis in Tokyo with the population curves of Culex tritaeniorhynchus is presented in Figure 17. In this instance, population curves have been constructed from horse baited trap collections and from human biting collections. From this graph it is apparent that a seven week interval separated the peak of mosquito population from the peak of the epidemic. However, reference to Figure 1 will indicate a twin peaked curve for Culex tritaeniorhynchus when light trap collections are used as the basis for constructing a population curve, with the first and highest peak occurring during the week of 29 July and with a secondary peak occurring during the week of 12 August.

Some interesting comparisons can be made with reference to the interval between the Culex tritaeniorhynchus population peak and the Japanese B encephalitis epidemic peak during the past four years in Tokyo. These intervals are listed below for the four year period together with the number of cases occurring in each of these years.

Year	No. of Cases	Interval Between Peaks
1948	1,959	3 weeks
1949	204	7 weeks
1950	1,304	3 weeks
1951	174	7 weeks

Data on mosquito populations in 1948 were taken from Kitaoka, Miura et al (17), while data for the succeeding three years was obtained by this organization. In the Annual Report for 1950 (1), reference was made to data provided by Petrakis on human biting collections in Yokohama during 1949. It was pointed out that Petrakis obtained a twin peaked curve by this method of collection for Culex tritaeniorhynchus during 1949, the first peak preceding the epidemic peak by 3 weeks. As pointed out previously, the curve for Culex tritaeniorhynchus obtained in Tokyo during 1951 by light trap differed somewhat from the curves obtained by human biting collection and by animal bait trap collection. These variables should not be overlooked in this analysis.

It can thus be ascertained that two entomological factors may possibly be involved as epidemic determinants. The first is mosquito population size (see Figures 1, 3, 4), and the second is the period of peak mosquito population and biting activity. A third entomological factor, degree of mosquito infectivity will be discussed later in this report. Our present state of knowledge of these factors is inadequate for evaluation of their importance.

During 1951 an attempt was made to correlate case rates of Japanese B encephalitis in the various kus (precincts) of Tokyo with the biting rates shown by Culex tritaeniorhynchus. The latter information was obtained from the human biting collection program mentioned above. Unfortunately, there was not an even distribution of students engaged in this collection program throughout the city. Case rates for the various kus are given in Table VIII together with the results obtained on Culex tritaeniorhynchus in the biting collection program. It will be noted that differences in case rates are very small and, similarly, there are no appreciable differences in the Culex tritaeniorhynchus biting rates. It will also be noted that the number of cases occurring in many of the kus is very small, and this was also true of the number of Culex tritaeniorhynchus taken in many of the kus. It is questionable if any valid conclusions can be obtained from this analysis.

In the four rural areas near Tokyo where human biting collections were undertaken, a similar comparison of Culex tritaeniorhynchus biting rates with case rates of Japanese B encephalitis was made. Four towns were involved in this study and each had 25 students participating in the biting collection program. Approximately 600 man-hours of collection were made in each town. The four towns are listed on the following page with their respective case rates and Culex tritaeniorhynchus biting rates.
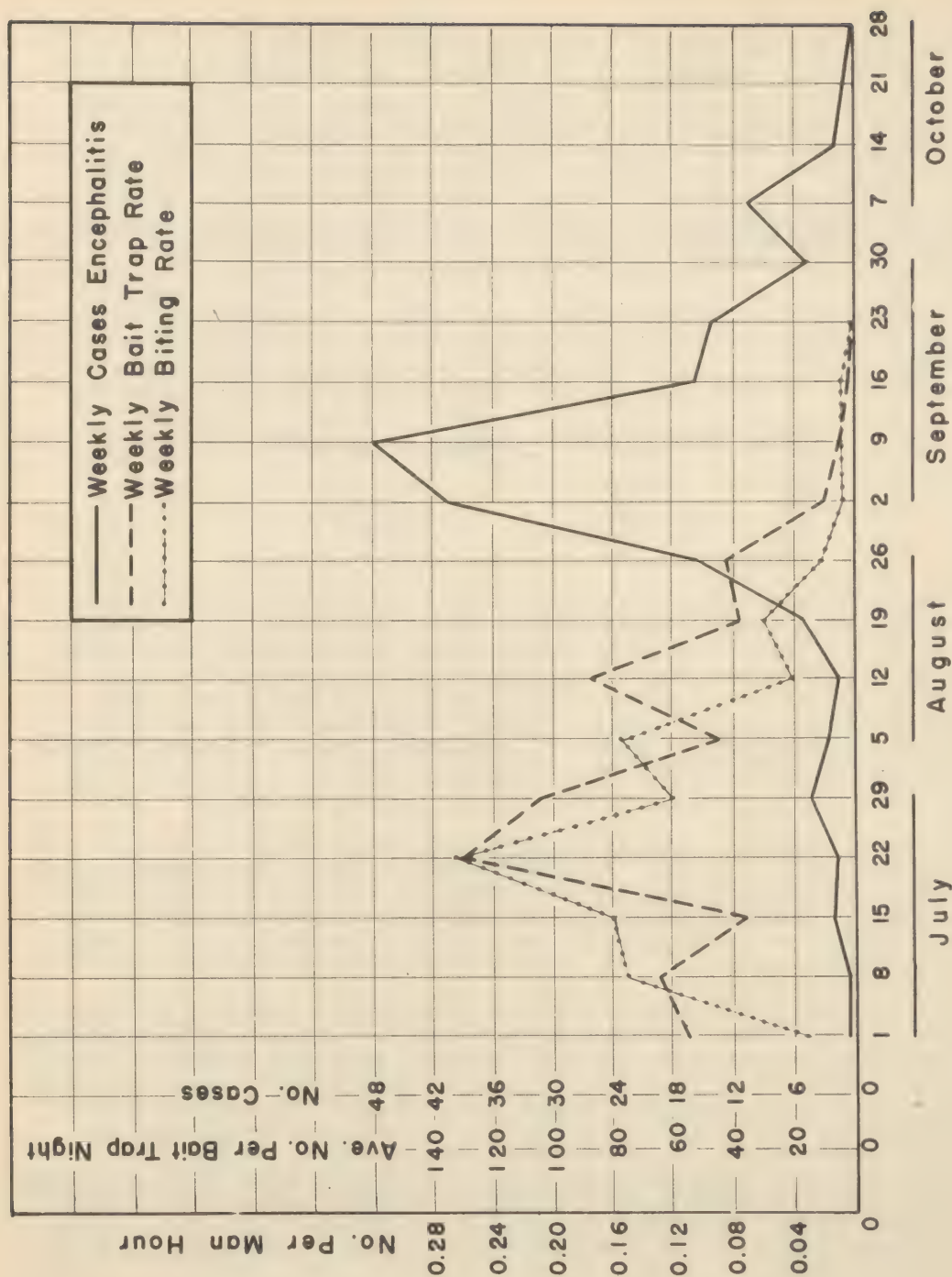
Figure 17. Relation of Culex tritaeniorhynchus to Tokyo JBE Epidemic of 1951

Town	Cases Per 100,000	C. tritaeniorhynchus Biting Rate Per Man-hour
Yatsuka	0	0.07
Jindai	0	0.18
Tachibana	4	0.13
Yamato	27	0.10

As in the analysis made for Tokyo during 1951, case occurrence was small and differences in biting rates similarly were small.

Table VIII. Relation of Cases to Biting Rates by Ku, Tokyo, 1951

Ku	No. Cases	Population (1 Sept 51)	Cases Per 100,000	C. tritaen. Biting Rate	No. Hrs. Biting Collect.	No. Students Collecting
Chiyoda	3	115,609	2.6	-	0	0
Chuo	4	165,768	2.4	0	22	1
Minato	4	230,851	1.7	-	0	0
Shinjuku	7	272,343	2.6	0.12	242	10
Bunkyo	0	204,248	0	0	22	1
Daito	2	278,403	0.7	-	0	0
Sumida	3	258,612	1.2	0.01	152	6
Koto	3	206,642	1.5	0.04	26	1
Shinagawa	6	313,157	1.9	0.04	26	1
Daiden (Ota)	18	438,442	4.1	0.04	26	1
Setagaya	24	433,938	5.5	0.25	171	7
Meguro	1	218,845	0.5	0.05	580	25
Shibuya	4	195,656	2.0	0.01	71	3
Nakano	3	233,850	1.3	0.06	313	13
Suginami	9	347,047	2.6	0.07	125	5
Toshima	8	237,218	3.4	0.08	125	5
Kita	11	290,078	3.8	-	0	0
Arakawa	3	218,872	1.4	1.04*	24	1
Adachi	11	238,240	3.9	-	0	0
Nerima	6	134,007	4.5	-	0	0
Katsushika	3	255,262	1.2	0.05	92	4
Edogawa	2	218,653	0.9	0.01	370	15
Itabashi	14	240,340	5.8	0	26	1
	149	5,791,081	2.6	0.08	2413	100

* All collected during July

RELATION OF CULEX TRITAENIORHYNCHUS POPULATION TO THE 1951 KANTO EPIZOOTIC: A comparison of the Culex tritaeniorhynchus population curves prepared from horse baited traps with the incidence of equine encephalitis in the Kanto area of Japan during 1951 is presented in Figure 18. Epizootic data are based on reported cases by approximate date of onset of symptoms. It will be noted that the two population curves do not correspond with reference to the peak week and consequently it is impossible to ascertain with certainty the interval between the peak of the C. tritaeniorhynchus as measured by these two methods, and the peak of encephalitis incidence in horses. Similar comparisons have been drawn for the population of this species with the incidence of equine cases in the Kanto area during 1949 and 1950 (11, 1). As in the two previous years, it has been necessary to compare mosquito data with the Kanto area epizootic rather than with cases in the Tokyo area due to the small size of the equine population of Tokyo. The Kanto area includes Tokyo and the six surrounding prefectures of

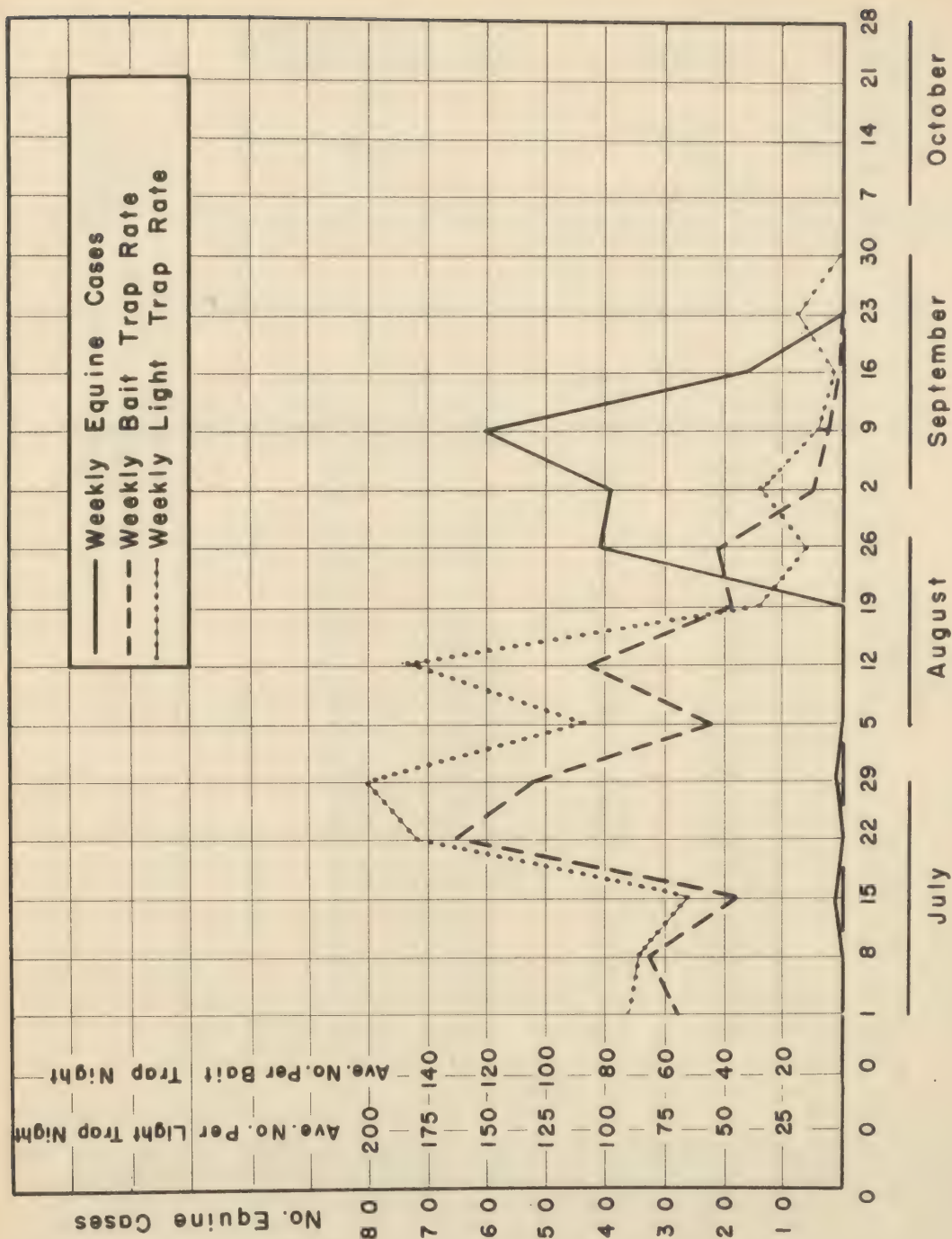Figure 18. Relation of Culex tritaeniorhynchus to 1951 Kanto Encephelitis Epizootic

Figure 19.   Incidence of Human and Equine Cases Encephalitis-Kanto Area, 1948

Figure 20.   Incidence of Human and Equine Cases Encephalitis-Kanto Area, 194
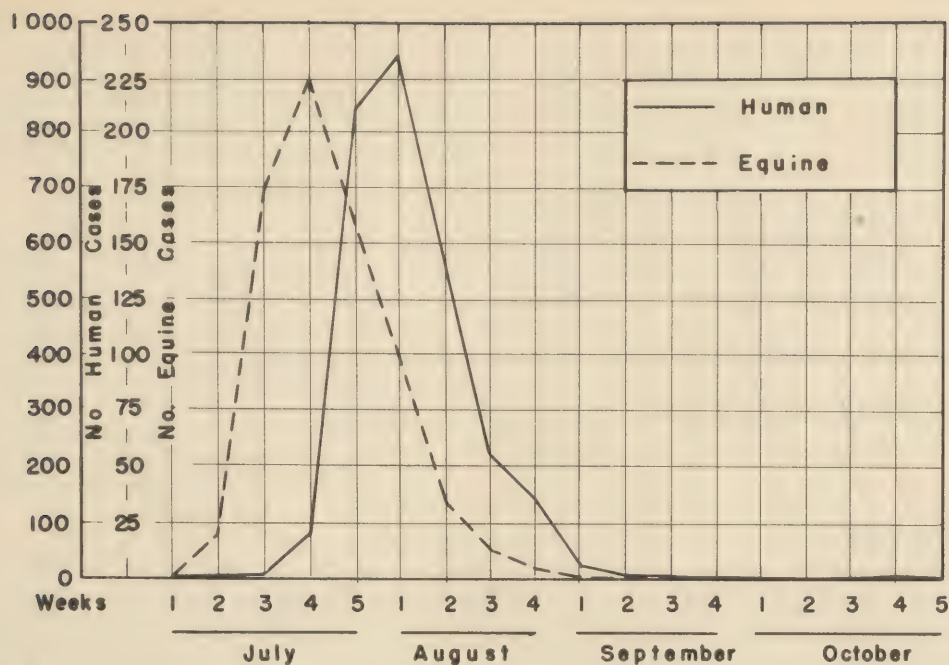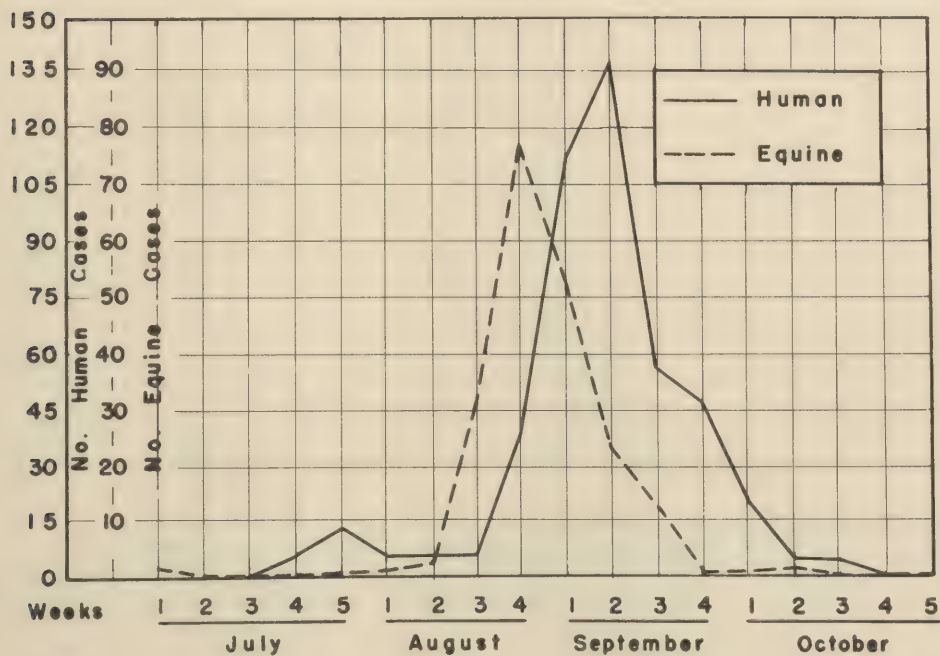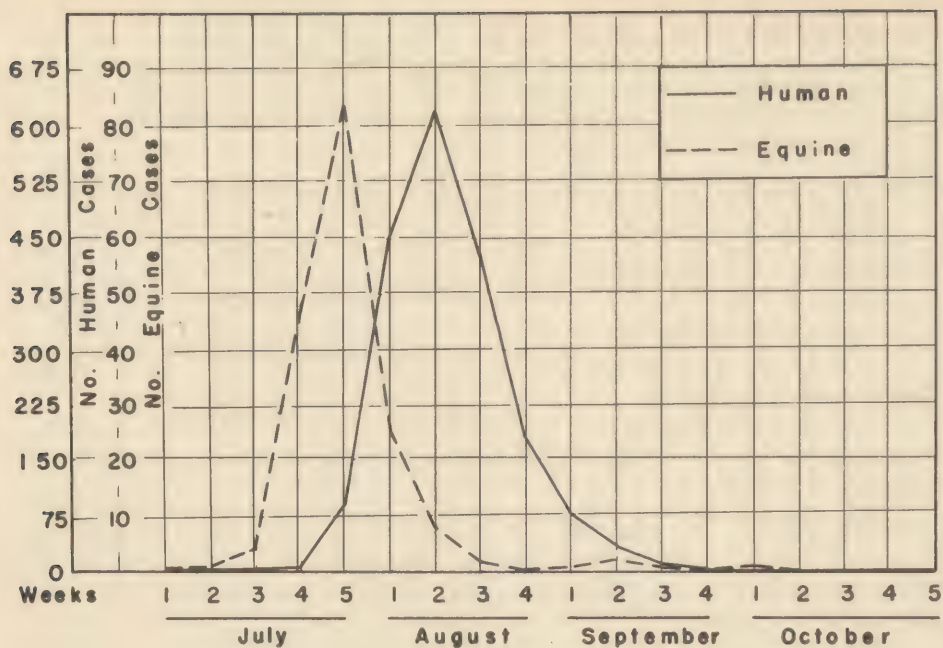
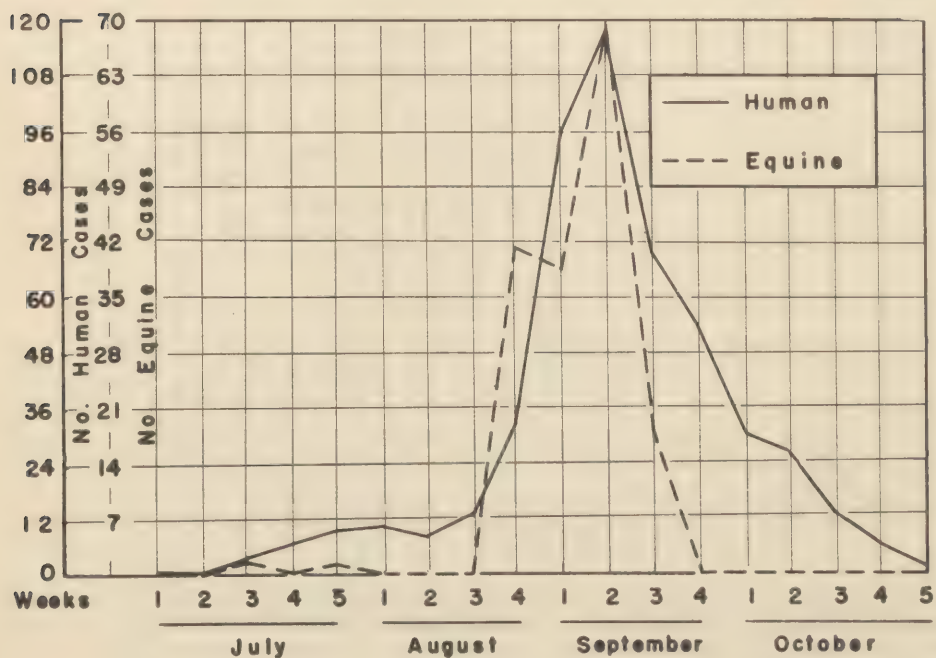Figure 21.   Incidence of Human and Equine Cases Encephalitis-Kanto Area, 1950

Figure 22.   Incidence of Human and Equine Cases Encephalitis-Kanto Area, 1951

Ibaraki, Tochigi, Gumma, Saitama, Chiba and Kanagawa. In 1949 (11), it was found that a five week interval separated the peak of the C. tritaeniorhynchus population, as measured by animal bait trap collections and by resting station collections, and the peak incidence of encephalitis in horses in the Kanto area. During the 1950 season (1), this interval was only one week. From Figure 18 it can be seen that the interval during the 1951 season was six or seven weeks.

Incidental to the correlation of mosquito population peaks with epidemic and epizootic peaks, it has been pointed out previously (1) that there appeared to be a constant two week interval between the peak of an epizootic and the peak of an epidemic, with the epizootic peak always preceding the epidemic peak. It was further pointed out that two possible hypotheses could be made from these observations: (a) that such a constant interval might indicate that epidemics stem directly from epizootics in horses, or (b) that such a constant interval might merely reflect differences in incubation periods of the disease in man and in horse. An examination of the weekly distribution of Japanese B encephalitis cases in Tokyo during 1951 in comparison with the weekly distribution of equine cases in the Kanto area during 1951 shows not a two week interval between the respective peaks, but two curves having concurrent peaks during the week beginning 12 September.

One criticism which can be levelled at these studies is that two non-comparable entities were being collated, since the epidemic data was limited to Tokyo while epizootic encompassed the much larger Kanto area. For this reason, we have collected epidemic data from the various prefectural health offices in the Kanto area for the four year period 1948-1951, and compared this information with the incidence of encephalitis in horses in the Kanto area during the same period. This data is presented graphically in Figures 19, 20, 21, 22. Data on the weekly incidence of encephalitis in horses was obtained from all prefectures for the four year period. Similar data was obtained for the weekly incidence of human cases from all prefectures for the four year period, except for Gumma prefecture, where only monthly incidence data was available. For this reason data from Gumma prefecture on both equine and human incidence has been excluded in Figures 19-22. From the data presently available on Gumma prefecture it is apparent that inclusion of this data would not have changed the configuration of the curves to any extent. All equine data used in these figures are based on reported cases by approximate date of onset of symptoms. All data on human cases are based on date of onset of symptoms. Data on human cases from Ibaraki, Tochigi, Chiba and Tokyo prefectures are clinically confirmed cases, while data from Saitama and Kanagawa prefectures are reported cases. Examination of Figures 19, 20, and 21 show a two week interval between the peak of epizootic incidence and the peak of epidemic incidence, with the epizootic peak occurring first. Figure 22, however, shows concurrent peaks in the incidence of the disease in man and in horses during 1951. Closer examination of the epizootic data during 1951 indicates that the greatest number of equine cases occurred in Ibaraki prefecture, in the northern part of the Kanto area, while examination of the epidemic data indicates the bulk of human cases were from Tokyo and Kanagawa (Yokohama) prefectures in the southern part of the Kanto area. By eliminating human cases from Tokyo and Kanagawa prefectures from the 1951 seasonal epidemic curve, a two week interval between epizootic and epidemic peaks can then be obtained. In Ibaraki prefecture itself, a two week interval separated epizootic and epidemic peaks in 1951. The elimination of equine cases from the northern part of the Kanto area, so that equine and human cases from the southern area could be compared, cannot be done since the number of remaining equine cases would be too small to warrant construction of a seasonal distribution curve.

VIRUS ISOLATION STUDIES ON MOSQUITO MATERIAL COLLECTED IN 1950 IN TOKYO: In the 1950 Annual Report (1) virus isolation studies on mosquito material collected in Tokyo during 1950 were described in detail. Subsequent to the writing of that report, a considerable amount of mosquito material collected during 1950 from the same sources was tested for the virus of Japanese B encephalitis, and a number of additional virus isolates were obtained. In the present report a summary is given on the results obtained from the entire collection of material. A tabulation of all frozen material

collected in Tokyo during 1950 and tested by the Department of Virus and Rickettsial Diseases and by the Department of Entomology of this laboratory is presented in Table IX.

Table IX. 1950 Mosquito Material Tested for Virus of Japanese B Encephalitis

Species	Fresh Material		Frozen Material	
	No. Lots	No. Mosquitoes	No. tubes	No. Mosquitoes
Anopheles hyrcanus sinensis	3	300	82	3,778
Armigeres subalbatus	18	1,369	55	1,714
Aedes vexans nipponii	7	511	85	4,098
Aedes togoi	0	0	2	25
Culex tritaeniorhynchus	61	5,963	988	85,140
Culex pipiens pallens	16	1,280	378	20,730
Culex bitaeniorhynchus	0	0	2	21
Total	105	9,423	1,592	115,506

The techniques used in this work were described in detail in the 1950 report (1). As an additional check against the possibility of contamination in laboratory procedures and as a check on the possibility of spontaneous or "natural" encephalitis in the stock mice, the Department of Entomology group undertook the running of control lots in the latter part of December 1950. A short time later, the group in the Department of Virus and Rickettsial Disease also undertook running control lots of mice. The procedure used in these control lots were as follows: Ten normal mice were taken at random from stock, placed in a cage and observed for symptoms for six days. If any central nervous system symptoms were noted, the brains from the affected mice were passed to a group of normal mice. After six days, if no symptoms were noted in a control lot, a blind passage was made. In a number of instances second blind passages also were made. During the period in which controls were run by the Department of Entomology, 38 isolations of Japanese B encephalitis were made from mosquito material but none were obtained from the controls. During this period 69 control lots were run, 101 passages made, and a total of 1690 mice used. The Department of Virus and Rickettsial Diseases also failed to obtain any isolates from control lots.

Two weeks after the control series was instituted, very careful screening of stock mice was begun. A total of 27,650 mice were examined, given a spin test, and from this number 204 sick mice were segregated and held for observation or taken for passage. No virus isolations were obtained from this source.

Because the number of isolates obtained in this study was so large, suspicion of possible contamination arose fairly early in the study. As a further check on the validity of the work being performed in this organization, a series of 24 tubes of Culex tritaeniorhynchus were selected and shipped to the Army Medical Service Graduate School, Washington, D.C. for testing for the presence of Japanese B encephalitis virus. All mosquitoes in this series had been collected in the field, identified, hermetically sealed and frozen on dry ice in a room in which no virus work had previously been performed. Twelve of the tubes were selected from "hot lots", i.e. lots from which other tubes previously tested had yielded virus isolates, and the remaining twelve tubes were selected from "cold" lots, i.e. lots from which other tubes had failed to yield isolates. From this material the Virus Laboratory of the Army Medical Service Graduate School recovered three virus isolates (18). These isolates were identified as Japanese B encephalitis by complement fixation test, by neutralization test and by haemagglutination-inhibition test. It was further stated (18) that preliminary results indicated an additional positive isolation had been obtained from one lot on the second attempt.

Isolates and Their Identification - In the 1950 Annual Report (1), 75 virus isolations were reported, and 74 of these isolates listed in Table XXII of that report. Subsequent to the writing of the 1950 report, 42 additional isolates were obtained, making a total of 117 for the entire project. The 43 isolates which were not previously listed in the 1950 report, are listed here in Table X. All isolates listed in this table were tentatively identified as JBE by cross-complement fixation test utilizing hyperimmune sera of Japanese B encephalitis, St. Louis encephalitis and Eastern Equine encephalitis or Western equine encephalitis. Complement fixation tests were conducted by the Department of Virus and Rickettsial Diseases. Titres for Japanese B encephalitis in the series of 117 isolates were as follows:

```
1:8   ......................   7
1:16  ......................  28
1:32  ......................  48
1:64  ......................  23
1:128 ......................  10
1:256 ......................   1
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A small number of the isolates obtained in this series are being further studied for positive identification by the Department of Virus and Rickettsial Diseases using neutralization and cross-protection tests.

Distribution of Isolates and Infectivity Indices - Distribution of isolates by source of collection is given in Table XI. It will be noted that the general distribution of isolates follows the general distribution of lots tested, with small discrepancies being found in the cases of horse and cow baited traps. Distribution of the isolates by date of collection is presented in Table XII, and from this we have calculated percentage isolation for each week during the 1950 season. In the 1950 Annual Report (1) we prepared indices of Culex tritaeniorhynchus infectivity for comparison with the course of the epizootic and epidemic of that year. These "indices of infectivity" were prepared from data on the 75 isolates which had been obtained to date. In Figures 23 and 24 we have corrected these infectivity indices by basing them on the results obtained from the entire series of 117 isolates. The resultant indices do not differ significantly from those prepared in the earlier report. The method of preparing such an index is as follows: the percentage infection in Culex tritaeniorhynchus is obtained for each week of this season as shown in Table XII. The weekly percentage infection factor is then multiplied against the corresponding weekly Culex tritaeniorhynchus collection rate from one of the population curves. After obtaining this index for each of the weeks in the season, all values are plotted and a curve drawn. The resultant curve is presumed to be a rough index of the size of the infective Culex tritaeniorhynchus population in nature. In Figure 23, the infectivity index has been prepared from the human biting collection curve and in Figure 24 a similar index has been drawn from the horse bait trap collection curve. These indices have then been compared with the actual course of the epidemic and epizootic. Both figures show striking correlation between the Culex tritaeniorhynchus infectivity indices and the course of the epidemic and epizootic. If a 4-5 day incubation is assumed in horses then the infectivity indices shown in Figures 23 and 24 can almost be superimposed upon the epizootic curve. It would appear from these figures that the epizootic stems directly from the infective Culex tritaeniorhynchus population, and that the epidemic also results from this infective mosquito population, but manifests itself at a later date due to a longer incubation period in man (1, 19).

In the 1950 annual report (1), a number of factors bearing upon the validity of the infectivity indices were discussed. To this list might be added the effect of dry ice storage on isolations of JBE virus from mosquitoes. The mosquito material used in this project was tested over a period of nine months. If storage of material over a length of time has any deleterious effect on isolations, the percentage isolation from such stored material might be considerably affected. An examination of available data to determine the effects of storage on virus isolation fails to give a positive indication of such effects, although superficially it appears that a small decrease in isolations occurred with the passage of time. If the assumption is made that storage reduced the rate of isolation in the 1950 mosquito material during the latter portion of

Table X. Additional Virus Isolates from Culex tritaeniorhynchus Collected
in Tokyo During 1950

Test No.	Coll. No.	Date Collected	Collection Locally	Habitat or Bait	No. Mosq.	Isolation By
E-820	413	15 July '50	Yoyogi B. T.	Horse	100	Entomology
E-678	426	17 July '50	Yoyogi B. T.	Horse	100	Entomology
FM-330	466	21 July '50	Yoyogi B. T.	Horse	100	Virus
FM-339	477	22 July '50	Hatagaya B. T.	Cow	100	Virus
E-939	481	24 July '50	Ueno Park B. T.	Horse	100	Entomology
E-895	490	25 July '50	Ueno Park B. T.	Horse	100	Entomology
E-1028	490	25 July '50	Ueno Park B. T.	Horse	100	Entomology
FM-356	495	25 July '50	Hatagaya B. T.	Cow	100	Virus
FM-365	519	27 July '50	Hatagaya B. T.	Cow	100	Virus
FM-370	520	27 July '50	Ueno Park B. T.	Horse	100	Virus
E-868	520	27 July '50	Ueno Park B. T.	Horse	100	Entomology
E-1034	520	27 July '50	Ueno Park B. T.	Horse	100	Entomology
FM-371	528	28 July '50	Hatagaya B. T.	Cow	100	Virus
E-1040	530	28 July '50	Yoyogi B. T.	Horse	100	Entomology
E-1075	530	28 July '50	Yoyogi B. T.	Horse	50	Entomology
E-1076	530	28 July '50	Yoyogi B. T.	Horse	50	Entomology
E-1118	530	28 July '50	Yoyogi B. T.	Horse	50	Entomology
E-1120	530	28 July '50	Yoyogi B. T.	Horse	50	Entomology
FM-380	531	28 July '50	Setagaya Race Track	Stable	79	Virus
FM-384	544	31 July '50	Yoyogi B. T.	Horse	100	Virus
FM-394	552	1 Aug. '50	Hatagaya B. T.	Cow	100	Virus
FM-407	563	1 Aug. '50	Yoyogi B. T	Horse	90	Virus
E-907	563	1 Aug. '50	Yoyogi B. T.	Horse	100	Entomology
FM-388	561	2 Aug. '50	Ueno Park B. T.	Pig	100	Virus
E-905	570	3 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-927	570	3 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-896	571	3 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-1023	571	3 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-1029	571	3 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-1035	571	3 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
FM-408	580	4 Aug. '50	Yoyogi B. T.	Horse	100	Virus
E-506	580	4 Aug. '50	Yoyogi B. T.	Horse	100	Entomology
E-1136	580	4 Aug. '50	Yoyogi B. T.	Horse	50	Entomology
E-1140	580	4 Aug. '50	Yoyogi B. T.	Horse	50	Entomology
E-508	581	4 Aug. '50	Hatagaya B. T.	Cow	100	Entomology
FM-409	582	4 Aug. '50	Setagaya Race Track	Stable	100	Virus
FM-412	583	4 Aug. '50	Shimotokaido	Dairy	109	Virus
FM-513	595	7 Aug. '50	Ueno Park B. T.	Cow	29	Entomology
FM-417	621	9 Aug. '50	Ikenoue	Dairy	100	Virus
FM-52	627	10 Aug. '50	Ueno Park B. T.	Horse	100	Virus
E-525	627	10 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-565	627	10 Aug. '50	Ueno Park B. T.	Horse	100	Entomology
E-572	685	18 Aug. '50	Shimotokaido	Dairy	100	Entomology

the testing period, this still would not materially affect the configuration of the in-
fectivity indices in Figures 23 and 24, since almost all the C. tritaeniorhynchus material
tested during the last three months of the project was collected during the two week period
around the peak of the mosquito population. Thus, if any isolates were lost in this mat-
erial the net effect of this introduced error would be a decrease in the magnitude of the
peak of the infectivity indices.

Table XI. JBE virus Isolates from Culex tritaeniorhynchus Collected
in 1950, by Source of Collection

Source	Lots Run No.	Lots Run %	Isolations No.	Isolations %
Horse baited traps	668	63	64	58
Cow baited traps	147	14	27	23
Pig baited traps	95	9	15	13
Dairies	78	7	7	6
Stables	39	4	3	2
Pig pens	20	2	1	1
Chicken houses	2	1	0	0
Total	1049	100	117	100

Table XII. Distribution of Virus Isolates from Culex tritaeniorhynchus
Collected During 1950, by Date of Collection

Week	No. Lots Run	No. Isolations	Percentage Isolation
4 June	1	0	0
11 June	2	0	0
18 June	4	0	0
25 June	7	0	0
2 July	38	1	3
9 July	103	4	4
16 July	284	9	3
23 July	255	35	14
30 July	185	35	19
6 Aug.	76	23	30
13 Aug.	37	9	24
20 Aug.	26	0	0
27 Aug.	18	1	6
3 Sept.	9	0	0
10 Sept.	3	0	0
17 Sept.	1	0	0
	1049	117	11

VIRUS ISOLATION STUDIES ON MOSQUITO MATERIAL COLLECTED IN 1951 IN TOKYO: For the third consecutive year, mosquitoes were collected in the Tokyo area and tested for the presence of Japanese B encephalitis virus. As in 1950, two departments in this laboratory, the Department of Virus and Rickettsial Diseases and the Department of Entomology, participated in this isolation work. Because the mosquito population was so much smaller in 1951 than in 1950, considerably less material was collected during 1951 than in the former year, and a total of only 18,548 mosquitoes was available for testing. This material is broken down by species in Table XIII. Techniques used by both departments were identical with those used in 1950 (1). Control lots of mice were run, as described in the previous section of this report, by both departments throughout the program. No natural infection was demonstrable.

From the material listed in Table XIII, 17 isolations of Japanese B encephalitis virus were obtained, 6 of these by the Department of Entomology and 11 by the Department of Virus and Rickettsial Diseases. These isolates are listed in Table XIV together with pertinent collection data. All isolations were obtained from Culex tritaeniorhynchus. Other species yielded entirely negative results for the third consecutive year. All isolates have been tentatively identified as Japanese B encephalitis by
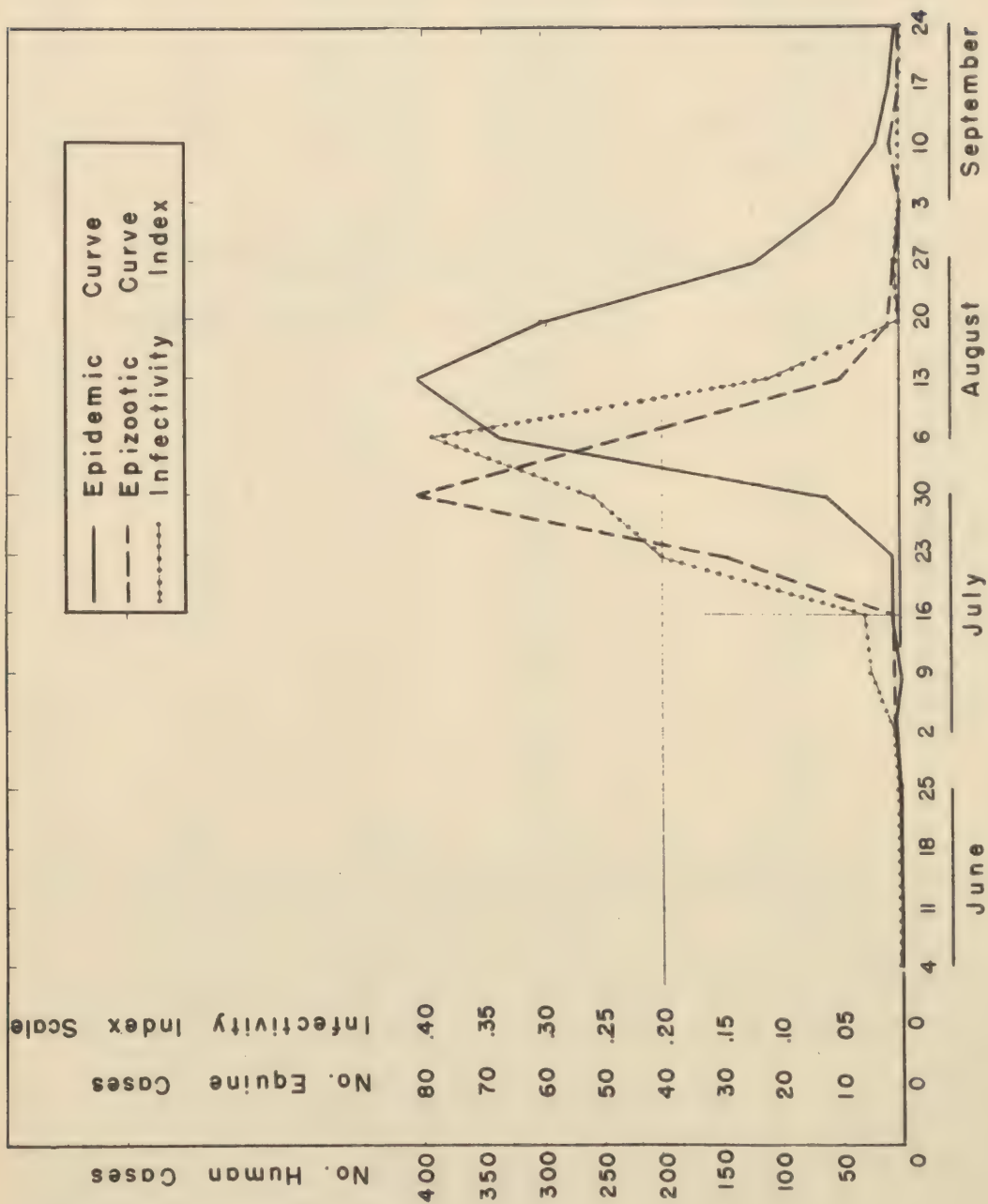
Figure 23. *Culex tritaeniorhynchus* Infectivity Index and the 1950 Epizootic and Epidemic of Japanese B Encephalitis (Based on Horse Bait Trap Collections)
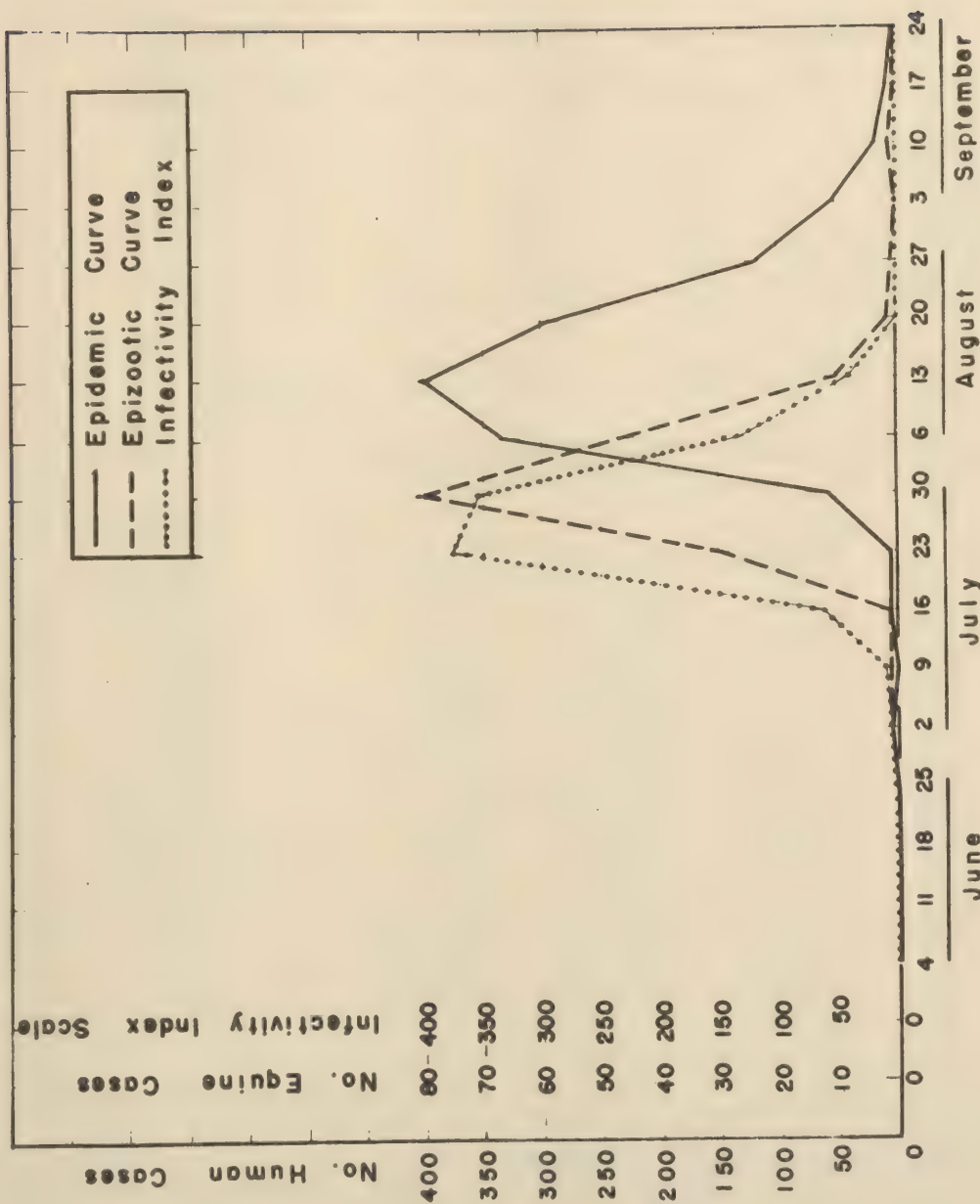
Figure 24. *Culex triteniorhynchus* Infectivity Index and the 1950 Epizootic and Epidemic of Japanese B Encephalitis (Based on Horse Bait Trap Collections)

complement fixation tests conducted by the Department of Virus and Rickettsial Diseases. Confirmation of these identifications by means of neutralization and cross-protection tests is to be accomplished.

Table XIII. Mosquito Material Collected in Tokyo During 1951
and Tested for JBE Virus

| | Entomology Dep't | | Virus Dep't | | Total | |
	No. Lots	No. Mosquitoes	No. Lots	No. Mosquitoes	No. Lots	No. Mosquitoes
Anopheles hyrcanus sinensis	7	113	*29	761	36	874
Armigeres subalbatus	19	372	0	0	19	372
Aedes albopictus	1	11	0	0	1	11
Aedes vexans nipponii	30	837	0	0	30	837
Culex pipiens pallens	154	6978	0	0	154	6978
Culex tritaeniorhynchus	81	4226	135	5251	216	9477
Total	292	12,537	164	6,012	456	18,549

Table XIV. JBE Virus Isolates from Culex tritaeniorhynchus Collected in Tokyo During 1951

Test No.	Coll. No.	Date Coll.	Coll. Locally	Habitat or Bait	No. Mosq.	Isolation By
1-177	375	15 Aug.	Yoyogi B. T.	Horse	100	Entomology
1-182	387	16 Aug.	Yoyogi B. T.	Horse	100	Entomology
M1-132F	403	18 Aug.	Yoyogi B. T.	Horse	96	Virus
M1-135F	406	20 Aug.	Yoyogi B. T.	Horse	50	Virus
M1-136F	418	21 Aug.	Yoyogi B. T.	Horse	41	Virus
1-189	437	23 Aug.	Yoyogi B. T.	Horse	88	Entomology
M1-143F	442	23 Aug.	Setagaya	Stable	12	Virus
M1-146F	451	24 Aug.	Tokyo Hort. Sch.	Pig Pen	17	Virus
1-186	455	25 Aug.	Yoyogi B. T.	Horse	47	Entomology
1-139	458	27 Aug.	Yoyogi B. T.	Horse	64	Entomology
M1-149F	463	27 Aug.	Ikenoue	Dairy	28	Virus
1-162	469	28 Aug.	Yoyogi B. T.	Horse	36	Entomology
M1-155F	473	29 Aug.	Yoyogi B. T.	Horse	80	Virus
M1-154F	475	29 Aug.	Yoyogi B. T.	Chickens	14	Virus
M1-158F	486	30 Aug.	Yoyogi B. T.	Horse	109	Virus
M1-157F	487	30 Aug.	Yoyogi B. T.	Chickens	44	Virus
M1-163F	502	1 Sept.	Yoyogi B. T.	Chickens	12	Virus

An examination of Table XV will indicate a predominance of isolates from mosquitoes collected in horse baited traps. In the work with the 1950 material (see Table XI), the distribution of isolates was generally proportionate to the volume of material tested from the various sources of collection. Whether the distribution shown in Table XV shows anything of significance, or whether it merely reflects a disproportionate distribution due to the small size of the 1951 isolate series, cannot be determined.

Distribution of the isolates from the 1951 collections by date of collection is shown in Table XVI and from this percentage isolation has been calculated, as was done in Table XII for material collected during the 1950 season. During 1951, isolates were obtained from material collected over a seventeen day span. The earliest

Table XV. JBE Virus Isolates from Culex tritaeniorhynchus Col-
lected in 1951, by Source of Collection

Source	Lots Run		Isolations	
	No.	%	No.	%
Horse baited traps	78	36	11	65
Bird baited traps	63	29	3	17
Stables	20	9	1	6
Pig baited traps	11	5	0	0
Bird observation blinds	11	5	0	0
Pig pens	9	4	1	6
Light traps	9	4	0	0
Dairies	8	4	1	6
CO_2 traps	6	3	0	0
Chicken houses	1	1	0	0
Total	216	100	17	100

Table XVI. Weekly Distribution of Virus Isolates from Culex tri-
taeniorhynchus Collected During 1951 by Date of Collection

Week	No. Lots Run	No. Isolations	Percentage Isolation
29 April	2	0	0
6 May	0	0	0
13 May	1	0	0
20 May	4	0	0
27 May	1	0	0
3 June	1	0	0
10 June	6	0	0
17 June	2	0	0
24 June	10	0	0
1 July	13	0	0
8 July	18	0	0
15 July	19	0	0
22 July	20	0	0
29 July	23	0	0
5 August	22	0	0
12 August	31	3	10
19 August	14	6	43
26 August	19	8	42
2 Sept.	4	0	0
9 Sept.	4	0	0
16 Sept.	0	0	0
23 Sept.	2	0	0
	216	17	8

collection date from which an isolate was obtained was 15 August, with the last iso-
late being obtained from a collection made on 1 September. While the largest number
of isolates were obtained from mosquitoes collected during the week beginning 26 Aug-
ust, the greatest percentage isolation was obtained in material collected during that
week and the previous week.

Comparison of Isolation Rates in 1950 and 1951 - A comparison of virus iso-
lation results from collections made in 1951 with those made in 1950 provides some

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interesting data. Virus isolation attempts on material collected in 1951 were conducted until 19 November 1951, by the Department of Entomology. From this material, six isolations of Japanese B encephalitis were made. This represents an isolation rate of 7.4% from all Culex tritaeniorhynchus material tested. During the same testing period of 1950, the Department of Entomology obtained an isolation rate of 6.0% from all C. tritaeniorhynchus tested. During 1950, 96.5% of the C. tritaeniorhynchus material referred to above was collected during the epidemic period or within the two weeks immediately preceding the epidemic, while in 1951 only 78% of the C. tritaeniorhynchus material was collected in the corresponding period. Techniques used during both years were identical and the same personnel performed the work. Thus, the results suggest that the smaller size of the 1951 epidemic, considering entomological factors alone, was due not to a reduced rate of infection in mosquitoes but due to the greatly reduced mosquito population. It is evident that further work over a period of several years would be necessary to establish whether infection rates in mosquitoes are constant or variable.

In Figure 25 comparison has been made between the 1950 and 1951 epidemic of Japanese B encephalitis in Tokyo and the corresponding infectivity indices for Culex tritaeniorhynchus during the same year. The weekly dates shown on the horizontal scale are for 1950 and data for 1951 is plotted for the corresponding weeks of that year. The infectivity indices were prepared, as described previously, by multiplying the percentage isolation for each week against the human biting collection rate for the corresponding week. It will be noted from Figure 25 that the magnitude and general configuration of the infectivity indices show a marked correlation with the magnitude and general configuration of the epidemic curves for the respective years. It should be noted, however, that while the peak of the 1950 infectivity index precedes the peak of the 1950 epidemic by one week, the period separating the corresponding peaks in 1951 is three weeks.

ATTEMPTS AT NATURAL TRANSMISSION OF JBE VIRUS TO MICE BY MOSQUITOES: In an attempt to determine whether or not mosquitoes trapped alive in nature could transmit JBE virus to white mice, a program was established during the 1951 season of placing white mice in bait traps each night traps were operated. Five mice, each immobilized in a wire holder, were placed in each bait trap late in the afternoon. The following morning they would be removed and held under observation for a period of 12 days. The brains of any mice showing central nervous system symptoms were then passed into another group of normal mice. This program commended on 11 July and was terminated on 7 September. A total of 131 lots of mice were placed in bait traps, 71 lots in bird baited traps, and 60 lots in horse baited traps. In three instances mice developed suggestive symptoms, but in each instance no virus was recovered. Of the 14 isolates obtained during 1951 from mosquitoes taken in bait traps, in all but one instance, mice had been placed in the bait traps. In none of these thirteen lots did any mice show central nervous system symptoms. While engorged mosquitoes were noted resting on the wire mouse holders on a few occasions, no evidence was obtained to indicate that mosquitoes actually fed on any of these mice.

As a second approach aimed at determining whether or not wild caught mosquitoes could infect mice with JBE virus, several attempts were made to feed wild caught mosquitoes on normal mice in the laboratory. Eleven such attempts were made utilizing batches of from 17 to 92 female mosquitoes during the period 17 July to 15 August. All mosquito lots contained Culex tritaeniorhynchus, except one which contained Anopheles hyrcanus sinensis. Two of these lots of mosquitoes were taken resting in stables and two lots were taken resting in dairies. All of these mosquitoes were engorged when taken, and had to be held in the laboratory until the blood meal was digested. Mortality during a two day holding period was exceedingly high, despite efforts at maintaining optimum temperature and humidity. An additional two lots of mosquitoes were taken from a bait trap in which a tank of liquid CO_2 had been placed, with the valve adjusted to permit a very slow discharge of gas which would serve as an attractant for mosquitoes. Four lots of mosquitoes were also taken from a bait trap in which
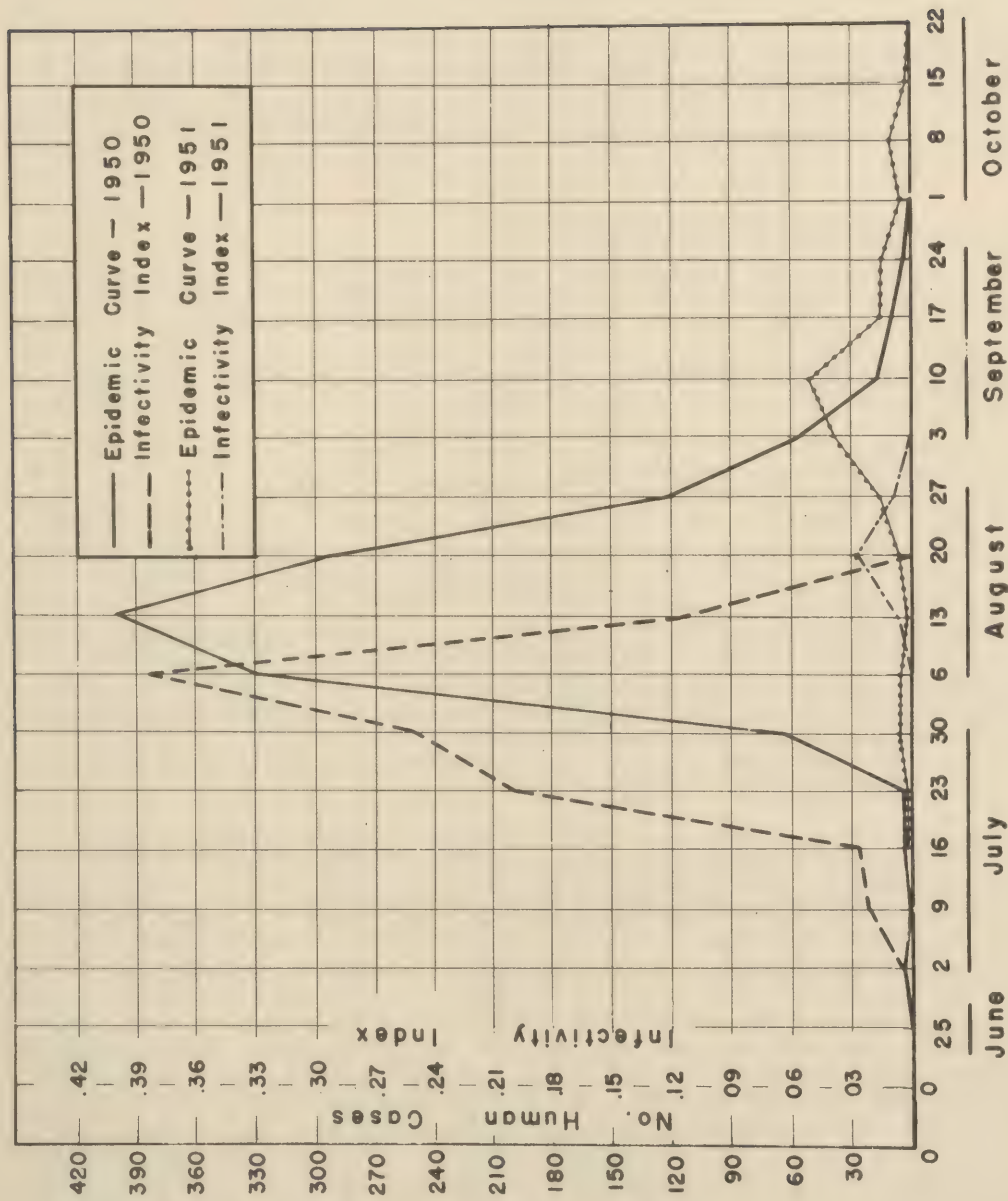
Figure 25. Infectivity Indices for *Culex tritaeniorhynchus* and the 1950 and 1951 Epidemics of JBE

a pig had been placed, entirely surrounded by wire screening. The last six lots, being unengorged, were offered mice as a blood meal without any holding period. In only one of the eleven attempts did mosquitoes engorge upon mice and in no instance was virus transmission demonstrated. The greatest difficulty encountered in this program was that of holding Culex tritaeniorhynchus adults alive, and because of our inability to solve this problem the program was discontinued after 15 August. For comments on the results of virus isolation tests on overwintering mosquitoes, see the following section of this report.

DISCUSSION OF THE VECTOR OF JAPANESE B ENCEPHALITIS: Leach, in his book entitled "Insect Transmission of Plant Diseases" (20), has stated four requirements which he considers the minimal requisition for adequate proof of insect transmission of a plant disease. Paraphrased, so that they apply to animal diseases, these rules would read as follows:

1. A close, although not necessarily a constant, association of the insect with diseased individuals must be demonstrated.

2. It must be demonstrated that the insect also regularly visits healthy individuals under conditions suitable for the transmission of the disease.

3. The presence of the pathogen or virus in the insect in nature or following feeding on a diseased individual must be demonstrated.

4. The disease must be produced experimentally by insect feeding or visitation under controlled conditions with adequate checks.

An examination of the data accumulated during the past three years on the relation of mosquitoes to the transmission of Japanese B encephalitis will show that evidence now weighs heavily toward the incrimination of Culex tritaeniorhynchus as the vector.

With respect to the first requirement listed above, it has been demonstrated that this species readily attacks man and other susceptible animals, and that the biting curve for the species closely approximates that of the epidemics and epizootics. Preparation of infectivity indices for this species for two years has shown a marked correlation between these infectivity indices and the course of epidemics and epizootics. The odds that this very close correlation should be due to chance alone seems entirely too small to affect their validity.

The configuration of the biting rate curves of Culex tritaeniorhynchus obtained from human biting collections and animal bait trap collections, together with the frequent demonstration of Japanese B encephalitis virus in this species prior to and during epidemics and epizootics of the disease should satisfy requirement 2. During the past two years, a total of 134 isolations of Japanese B encephalitis virus have been made from lots of Culex tritaeniorhynchus. This should amply fulfill requirement 3.

Evidence was presented in the 1949 Annual Report (11) to show that on eight occasions transmission of Japanese B encephalitis virus to young mice was accomplished with Culex tritaeniorhynchus. In seven of these successful transmissions, mosquitoes were infected on blood virus suspensions, while in the eighth mosquitoes were fed on an infected chicken. Since these transmissions were made to mice rather than to man and since, in most of these tests highly concentrated blood virus suspensions were used for infection of mosquitoes, it might be argued that the above evidence is inadequate to fulfill the requirements of rule 4. However, the severity of the symptoms of this disease, the high mortality rate and the lack of specific therapy prohibit the use of humans in transmission experiments. In view of this, it would seem reasonable to accept fulfillment of this last rule by the successful transmission of the virus from infected mice to healthy mice and by a similar demonstration in some other susceptible animal such as monkey, horse, or pig. Successful transmission of the disease to any of the above animals by wild caught mosquitoes would also be desirable.

The possible role of other mosquito species as vectors of Japanese B encephalitis is highly questionable. Mosquito population studies and virus isolation studies undertaken at this laboratory have failed to produce any evidence tending to incriminate any species of mosquito, other than Culex tritaeniorhynchus, as a vector of Japanese B encephalitis. While experimental transmission studies undertaken at this laboratory and by a number of other workers, have demonstrated that a large number of mosquito species occurring in Japan and elsewhere are capable of transmitting the virus after artificial infection, it must be borne in mind that in almost all such experimental transmissions, mosquito infection has been accomplished by feeding massive doses of blood virus suspensions. Until such time as it can be demonstrated that these species are capable of transmitting the disease from infected to healthy animals, and until positive confirmation of the occurrence of the virus in such mosquitoes in nature is obtained, it must be considered that the evidence necessary to incriminate any species, other than Culex tritaeniorhynchus, as a vector is entirely inadequate.

OVERWINTERING AND EARLY SEASON STUDIES ON MOSQUITOES: For the past three weeks overwintering studies on mosquitoes have been undertaken with the objective of determining how Culex tritaeniorhynchus overwinters. Many such surveys have been made by Japanese and American workers, but to date no one has succeeded in finding this species in any stage of its life history during the winter. Knowledge of how Culex tritaeniorhynchus overwinters would be of more than mere academic interest. The possibility that this species might harbor the virus of Japanese B encephalitis over the winter cannot be ruled out at present.

During January, February and March of 1951, a total of 64 collections of adult mosquitoes were made. During the latter part of April and early May light traps were operated 13 times and horse-baited traps 9 times in an effort to obtain early emerging C. tritaeniorhynchus adults for virus isolation studies. Three of the light traps yielded a total of 8 mosquito adults including 5 C. tritaeniorhynchus and 1 female C. tritaeniorhynchus was taken in the bait traps. The first catch of C. tritaeniorhynchus was made by a light trap placed inside a stable on 25 April. From the overwintering and early season collections a total of 8,809 adult mosquitoes were taken. A summary of overwintering adult mosquito collections, including light trap and bait trap collections made in April is presented in Table XVII.

Table XVII. Collections of Overwintering Adult Mosquitoes

Type Habitat	No. Collections	Culex pipiens	Culex hayashii	Culex orientalis	Culex tritaeniorhynchus	Totals
Caves, bomb shelters	24	1864	2467	20	0	4351
Basements, cellars	15	1851	3	7	0	1861
Houses, temples & bldgs.	9	1276	17	15	0	1308
Sheds	3	65	0	0	0	65
Dairies	3	11	0	0	0	11
Stables	2	8	0	0	0	8
Manholes, sewers	2	497	0	0	0	497
Tombs	2	82	4	0	0	86
Tunnels	2	565	0	1	0	566
Chicken houses	1	1	0	0	0	1
Other animal shelters	1	47	0	0	0	47
Light traps	3	3	0	0	5*	8
Total	67	6270	2491	43	5*	8809

* Taken in late April

During January, February and March of 1951, 27 larval collections were also taken, from which 1,023 larval identifications were made. These are summarized in Table XVIII. It will be noted that no Culex tritaeniorhynchus were taken.

Table XVIII. Collections of Overwintering Larval Mosquitoes

Species	Artificial Containers	Sewers	Cave Bottoms	Sumps	Totals
Aedes togoi	351	0	135	0	486
Aedes japonicus	70	0	0	0	70
Aedes albopictus	73	0	0	0	73
Culex pipiens	0	44	5	0	49
Uranotaenia bimaculata	150	0	86	0	236
Anopheles lindesayi	0	0	79	30	109
Totals	644	44	305	30	1023
No. Collections	18	2	6	1	27

All adult material taken during the winter and spring was tested for the presence of Japanese B encephalitis but with entirely negative results.

PRECIPITIN TESTS ON MOSQUITO BLOOD MEALS: In the 1950 Annual Report of this organization (1) a detailed discussion was given of precipitin testing of mosquito blood meals taken from mosquitoes collected during 1949 and 1950. The material tested had been collected almost exclusively from adult resting stations. During 1951 a series of smears was prepared from mosquitoes taken largely from animal bait traps and light traps. The objective in testing material from animal bait traps was to determine what proportion, if any, of the engorged mosquitoes taken in such traps would contain blood other than that of the bait animal. Engorged mosquitoes were collected in four light traps, two of which were operated inside stables, one on the grounds of a zoo, and the fourth on the grounds of a bird sanctuary. Only one engorged specimen was taken in the last named trap. A small series of smears also was taken from material collected in observation blinds at a bird sanctuary. Details on the preparation of smears, the preparation of antisera and the techniques used in the test have been given in the 1950 Annual Report (1). Techniques and standards used in 1951 were identical with those used in 1950.

A total of 2,417 smears from 13 species of mosquitoes were tested in 1951. Table XIX indicates the results obtained from this material based on the method or source of collection. It will be noted that the great majority of smears made from mosquitoes taken in animal bait traps contained the blood of the host animal, but that small percentages of smears did yield other blood types. The high percentage of equine positives obtained from light trap material can be explained by the fact that most of the material was obtained from light trap operated inside stables.

Table XIX. Summary of Precipitin Tests by Method of Collection

Collection Method	Human	Avian	Bovine	Goat	Equine	Porcine	Mixed	Negative	Totals
Horse baited traps	3	23	2	0	1152	10	5	11	1206
Bird baited traps	26	670	1	0	21	8	1	14	741
Pig baited traps	0	1	0	0	1	35	0	0	37
Light traps	5	19	0	3	258	8	1	2	296
Observation blinds	8	121	1	0	0	0	0	7	137
Totals	42	834	4	3	1432	61	7	34	2417

A better analysis of precipitin results can be obtained from a comparison of Tables XX and XXI. Table XX provides a breakdown of mosquito material by species and by method or source of collection, while Table XXI indicates the results obtained by precipitin test for each of the species tested. In general, it is found that the results obtained follow rather closely the source distribution of material. Thus, of 197 Anopheles hyrcanus sinensis taken from horse baited traps, 196 yielded equine positives. In the case of Aedes vexans, 265 smears were prepared from mosquitoes taken in horse baited traps and 116 prepared from mosquitoes taken in light traps (placed mainly in stables), a total of 381. It will be noted that there were 369 equine positives obtained from Aedes vexans smears. Or again, 348 smears were prepared from Culex pipiens collected in bird baited traps and an additional 108 smears prepared from specimens of this species taken in observation blinds at the bird sanctuary. The number of avian positives obtained from Culex pipiens was 429. These results parallel closely the results obtained in 1950 (1) in demonstrating that precipitin test results correspond in general with the source, place or method of collection. For this reason, precipitin surveys to determine the feeding habits of mosquito species are greatly influenced and easily biased by the scope and methods of collection.

Table XX. Species Distribution by Method of Collection-Precipitin Test Series

Species	Horse Traps	Bird Traps	Pig Traps	Light Traps	Observ. Blinds	Totals
Anopheles hyrcanus sinensis	197	3	0	7	0	207
Armigeres subalbatus	142	12	11	0	0	165
Aedes vexans nipponii	265	7	7	116	0	395
Aedes togoi	3	0	1	0	0	4
Aedes japonicus	5	0	0	0	0	5
Aedes nipponicus	1	0	0	0	0	1
Aedes albopictus	7	1	0	0	0	8
Culex tritaeniorhynchus	530	370	17	172	26	1115
Culex pipiens pallens	35	348	1	0	108	492
Culex bitaeniorhynchus	16	5	0	0	1	22
Culex mimeticus	0	1	0	0	0	1
Culex hayashii	0	0	0	0	1	1
Culex vorax	0	0	0	0	1	1
Totals	1201	747	37	295	137	2417

Table XXI. Summary of Precipitin Tests by Species

Species	Human	Avian	Bovine	Goat	Equine	Porcine	Mixed	Neg.	Totals
Anopheles hyrcanus sinensis	0	4	0	0	196	2	3	2	207
Armigeres subalbatus	0	12	0	0	142	10	0	1	165
Aedes vexans nipponii	4	12	1	0	369	7	1	1	395
Aedes togoi	0	0	0	0	3	1	0	0	4
Aedes japonicus	0	0	0	0	4	0	0	1	5
Aedes nipponicus	0	0	0	0	1	0	0	0	1
Aedes albopictus	1	0	0	0	6	1	0	0	8
Culex tritaeniorhynchus	17	370	1	3	663	37	3	21	1115
Culex pipiens pallens	20	429	2	0	31	3	0	7	492
Culex bitaeniorhynchus	0	5	0	0	17	0	0	0	22
Culex mimeticus	0	1	0	0	0	0	0	0	1
Culex hayashii	0	0	0	0	0	0	0	1	1
Culex vorax	0	1	0	0	0	0	0	0	1
Totals	42	834	4	3	1432	61	7	34	2417

ORGANIZATION AND ACTIVITIES OF THE ECOLOGY SECTION AT OMIYA: The Ecology Section of the Entomology Department was activated in June 1951 for the purpose of investigating problems of immediate interest in medical entomology, with primary emphasis of the role of arthropod vectors in the epidemiology of Japanese B encephalitis. Personnel initially assigned to this section consisted of two officers on TDY with Far East Command. In September one officer was detailed to the Taxonomic Entomology Section and the work of the Ecology Section was continued by one officer and one enlisted man.

Details of previous investigations of Japanese B encephalitis by this laboratory have been presented in this and in previous annual reports. It now appears well established that this disease is arthropod-borne, and investigations carried out by this laboratory and other workers during the past three years have served to focus attention most sharply on one species of mosquito, Culex tritaeniorhynchus. However, even if the evidence pointing toward this species as the vector responsible for transmission of the disease to man was accepted as conclusive, there still remain many unsettled problems. The more important details of information needed for an understanding of the epidemiology of Japanese B encephalitis include knowledge of the natural reservoir of the virus, the means by which this disease is perpetuated in the normal hosts, and positive knowledge of the vector or vectors responsible for the transmission of the disease to man. After an evaluation of the information on hand the following investigational program was outlined as the mission of the Ecology Section:

1. A study of the bionomics of suspected mosquito vectors, especially Culex tritaeniorhynchus.

2. Virus transmission tests, using experimental animals and suspected mosquito vectors.

3. A continued search for the virus of Japanese B encephalitis in field collected arthropods, particularly mosquitoes and mites.

4. Evaluation of the role, if any, of rodents and other small wild mammals in the epidemiology of Japanese B encephalitis.

Since insectary and laboratory facilities adequate for mosquito culturing were not available in Tokyo, it became necessary to establish these facilities in space made available at Camp Omiya, approximately 20 miles northwest of Tokyo. Two large rooms were constructed, in a permanent type building; one for office and general laboratory space, the other for an improvised insectary including a large built-in cage 8 x 10 x 13 feet. Minimal facilities were completed and occupied in August; additional equipment such as tables, smaller cages, rearing pans, and heating devices were added during the ensuing two months. Temperature control is limited to the capabilities of manually operated heaters. Humidity regulation is by the use of evaporation from pans and moistened fabrics.

Pending availability of an insectary and laboratory, field observations of the local mosquito fauna were conducted in the Tokyo area, and to a very limited extent in the vicinity of Kyoto. At both locations note was made of the relative scarcity of Culex tritaeniorhynchus, which has been discussed earlier in this report. Initially larvae of C. tritaeniorhynchus were collected from rice paddies but by 10 August most of these were dry. Later larvae were found in artificial containers. In many cases it was noted that heavy populations of Culex tritaeniorhynchus were associated with surface vegetation growth, especially with duckweed and green algae. Even the initially low larval population had disappeared in the Omiya area by 4 September. Large numbers of immature and adult stages of several species of mosquitoes were collected in routine field work but colonization was attempted with only three species: Culex tritaeniorhynchus, Culex pipiens, and Aedes albopictus.

Culex tritaeniorhynchus - Initially, a small cage was used for the emergence of adults of this species but heavy mortality required a change to the large built-in

cage as soon as it was completed. Mortality continued to be heavy, most adults living only 24-48 hours. This mortality appeared to be due, at least in part, to efforts to escape, if we may judge by the spatial distribution of the dead adults. Escape movements were almost uniformly toward the morning sunlight. Furthermore, adult mortality appeared to be highest during periods of low humidity. Peak populations were 700 on one occasion and 1500 a few days later, but even under these crowded conditions no mating swarms were observed. There was no oviposition and no engorged females were found in the cage. Females were not noted feeding on the rabbits or guinea pigs kept in their cage, nor did they bite the laboratory workers who were in the cage for varying periods during the day and evening. Because of the strong evidence against this species as a vector of Japanese B encephalitis attempts at colonization will be continued when wild stock is again available early in the spring. Search for the overwintering form has been unsuccessful which lends further impetus to colonization as an aid in solving the apparent disappearance of this species from Japan during the winter.

Culex pipiens - A colony of this species was established in a small cage in August. When the last of the Culex tritaeniorhynchus adults had died, the colony was transferred to the large built-in cage and it has thrived there. The average population has been 1000 adults. Small mating swarms have been present in the cage every morning and oviposition has been steady. Females have fed freely on rabbits, guinea pigs and chickens; especially heavy feeding was noted when a rabbit was immobilized with Sodium pentathol for three or four hours in the cage. This species, while not a strong suspect as a natural vector of Japanese B encephalitis, is very suitable for virus transmission studies because of its hardiness, longevity, and prolific reproduction in captivity. In addition its eggs will provide food for colonies of mites to be raised in connection with studies on Epidemic Hemorrhagic Fever.

Aedes albopictus - The colony was originally established in a screened wash tub, and later was transferred to a large cage. From the first, females fed avidly on humans, mice, guinea pigs and rabbits. Well defined swarms were noted mostly in the early morning hours and copulation was frequent all through the day, some pairs continuing to mate while the female took a blood meal. Eggs were deposited in large numbers on the tub walls and later in bamboo sections placed in the cage. Virus of Japanese B encephalitis has not been isolated from this species in studies by this laboratory, but the number of lots tested has been small. Furthermore its biting habits, distribution and container breeding habits make it somewhat suspect. The eggs of related species have been used as food for mite colonies. The laboratory colony was destroyed in November, probably through low humidity and through the presence of many mites in the breeding cage and on the adults. The mites have been identified as Tyroglyphus longior var. castellanii Hirst.

Other Activities - During the last three months of the year 53 birds of various species were examined for arthropod ectoparasites. Mites and Mallophaga recovered were shipped to the Taxonomic Entomology Section at Kyoto. Preliminary work was undertaken in the colonization of mites of various species to be used in the study of the epidemiology of Epidemic Hemorrhagic Fever. Quantities of mosquito larvae, pupae and adults, both field collected and reared, were sent to the Distribution Center for Parasitological Specimens, Army Medical Service Graduate School. Several lots of Phthirus pubis were sent to the same agency.

INVESTIGATIONS ON THE RESISTANCE OF LICE TO DDT: Early in 1951, reports were received from several army organizations in the Far East Command that DDT dusts were not producing the expected degree of control on human body lice. Concurrently with these reports numerous samples of 10% DDT dust were submitted to the Chemistry Department of this organization for chemical assay. A visit by a member of the Entomology Department to one of the areas where large scale dusting was underway confirmed that dust application was being done very thoroughly and that the rate of application was equal to or in excess of the usual recommended dosage ($1\frac{1}{2}$ to 2 oz. per uniform). It was also apparent from this visit that the degree of louse control was indeed inadequate.

These observations were most unusual since the efficacy of DDT in controlling human body lice had been well established both by laboratory studies and field tests. Bushland, McAlister et al (21, 22) found that in 24 hours 10% DDT produced 100% mortality in human body lice in beaker tests, in arm-and-leg tests, and in tests on grossly infected individuals. They further found that the duration of effectiveness of a 10% DDT powder was between 30-40 days after application. Studies in the field (23, 24, 25, 26, 27, 28) attest to the success of DDT dusts in combatting lice and epidemic typhus in many areas of the world. In view of the disparity of observations in the Far East Command with those made in other areas of the world, and because of the importance of the problem in relation to the health of the United Nations forces and to prisoners of war, a special program of investigation was undertaken by the Chemistry and Entomology departments of this laboratory.

Chemical analysis of DDT samples of technical grade and prepared dusting powder was carried out according to the method of the American Association of Official Agricultural Chemists (29). Stocks for evaluation were of three categories: (a) Old American DDT manufactured during or prior to 1945, (b) New American DDT manufactured in 1951, and (c) New Japanese DDT manufactured in 1951.

Some samples of Old American DDT examined were from old barrels which were rusty and no longer waterproof. In a number of cases, the samples were wet and no longer usable for dusting purposes. Such samples were dried before being assayed. Other samples of old stocks, packaged in individual four-ounce cans, were generally dry and uniformly powdered even though the cans in some cases were corroded.

Results of chemical assay of the three categories of samples are shown in Table XXII. As may be seen from this table, chemical assay of the DDT samples showed a low value of 7.5% DDT content. This low value lies well within the range for effective control, as reported by Bushland et al (22) who reported that powders containing as little as 1% DDT usually gave complete kill of lice within 24 hours for periods of eight to ten days after application.

Variation in the assay values of the various samples of Old American stocks had no apparent correlation with the condition of the samples when collected or delivered to the laboratory. Samples which required drying from a gummy state assayed higher in some cases than samples which were in apparent good condition and satisfactory for dusting purposes. However, by comparison with newer American samples and with Japanese manufactured dusting powders, the Old American samples were generally noticeably coarser and in many cases were discolored, apparently the result of rust from the cans and containers being transferred to the powder.

The various samples were also examined for particle size by screening samples through United States Standard Mesh sieves. Results of the examinations are shown in Table XXIII.

Specification MII-1180A, dated 7 February 1950 (30), which states the requirements of a suitable dusting powder, requires the insecticide powder to contain not less than 9.5% or more than 10.5% DDT by weight. The finished insecticide powder shall be a fine free-flowing material, devoid of lumps, having such fineness that not less than 99% shall pass a U. S. Standard No. 80 (177 micron) sieve (dry test). As will be pointed out later in this report, experiments with the powders of varying fineness did not show any apparent correlation between particle size and percentage kill of lice.

Investigation of Biologic Activity of DDT - All lice used in these tests were collected from vagrants in Tokyo. To the best of our knowledge, these lice strains had not been previously exposed to DDT, at least not for several months. An initial group of 18 petri dish tests were conducted in which lice were exposed to the insecticide for 24 hours and given no opportunity to feed. The test apparatus consisted of two discs of cloth treated with DDT, fitted snugly into the bottom of a petri dish. Ten or 20 lice were introduced into each dish and the top was placed over it. Only adult and

Table XXII. Chemical Assay of DDT Samples*

DDT Percentage	Dusting Powder Samples		
	#1	#2	#3
7.5-8.0	2	-	-
8.1-9.0	7	-	-
9.1-10.0	20	4	2
10.1-11.0	13	5	5
11.1-12.0	3	-	-
12.1-12.8	-	1	-
Total	45	10	7

DDT Percentage	Technical DDT Samples		
	#1	#2	#3
96.0	1	-	-
99.9	1	-	-
100.0	-	3	-
Total	2	3	0

Table XXIII. Particle Size of DDT Samples*

Percentage Sample Passing Sieve	Mesh and Approximate Particle Size								
	80 mesh (177 micra)			100 mesh (149 micra)			325 mesh (44 micra)		
	#1	#2	#3	#1	#2	#3	#1	#2	#3
0-40	2	-	-	11	-	-	32	-	-
41-70	9	-	-	9	-	-	4	-	-
71-80	5	-	-	9	-	-	-	2	-
81-90	7	-	-	5	-	-	-	1	1
91-95	10	1	-	2	1	1	-	-	2
96-98	2	-	-	2	1	-	2	2	1
99-100	3	4	5	-	3	4	-	-	1
Total	38	5	5	38	5	5	38	5	5

Legend for Tables XXII and XXIII

#1 - Old American DDT dusting powder manufactured during or prior to 1945.
#2 - New American DDT dusting powder manufactured during 1951.
#3 - New Japanese DDT dusting powder manufactured during 1951.
 * - By Department of Chemistry

third instar nymphs were used. DDT was applied by means of a dusting tower modified after Waters (31). The rate of dust application was 13 mg. per square inch (equivalent to 1½ oz. per uniform). Controls consisting of an identical arrangement, except for the substitution of the talc for the insecticide, were run with all tests. All tests were kept in desiccating jars, inside an incubator, at a constant temperature of 30°C. and at a constant relative humidity of 60%. Controls were kept in a second incubator in the same manner.

A second series of 52 tests on DDT samples was conducted using a modified procedure. In this series, gauze-covered pill-boxes were substituted for petri dishes, and lice were given an opportunity to feed by placing the boxes, gauze side down, on a human volunteer twice daily. Twenty adult lice were used in each of these tests. Controls were run concurrently with these tests, with all other procedures being identical to those outlined above.

A third series of tests, consisting of six insecticides other than DDT, were conducted using the pill-box feeding method.

Twelve samples of DDT were used in these tests. These samples could be classified as follows:

> Old American manufactured DDT .... 5 samples
> Newly manufactured American DDT .... 4 samples
> Newly manufactured Japanese DDT .... 3 samples

The first group of samples was from old World War II stocks at least five years old. No information was available on date of manufacture, name of manufacturer or procurement source. Four samples were taken from large drums, and the fifth was taken from a lot of two-ounce cans. The second group of four samples was obtained from stocks of newly manufactured DDT from four American manufacturers. The third group consisted of newly manufactured stocks from three Japanese manufacturers. None of the samples in the latter two groups were over three months old. All samples were ostensibly 10% DDT dusts. Assay data, particle size data, and diluent for the samples used are given in Table XXIV. All DDT assays and sieve tests were performed by the Department of Chemistry, 406th Medical General Laboratory.

Table XXIV. Characteristics of DDT Samples Tested*

Sample No.	DDT Assay	Pass Through 80 Mesh Sieve	Diluent
"Old American"			
#1	11.4%	70%	Talc
#2	9.4%	80%	Talc
#3	8.5%	88%	Talc
#4	11.1%	84%	Talc
#5	10.0%	99%	Prophyllite
"New American"			
#1	12.8%	100%	Talc
#2	10.9%	100%	Talc
#3	9.4%	100%	Prophyllite
#4	9.3%	100%	Prophyllite
"New Japanese"			
#1	9.7%	100%	Talc
#2	10.1%	100%	Talc
#3	9.8%	100%	Talc

* By Department of Chemistry

The first series of 18 tests was conducted over a 24-hour period without feeding the lice. Three samples of "Old American" DDT were used. The mortality results obtained in these tests are given in Table XXV. It will be noted that none of the materials tested produced over 60% mortality, and that all controls also exhibited high mortalities.

A second series of 52 tests in which all DDT samples were tested is summarized in Table XXVI. In this series, lice were held for 48 hours and given the opportunity to feed on a human twice daily. Mortalities have been indicated for the three groups

of DDT tested. It will be noted that the greatest mortality occurred when samples of American manufactured DDT were tested and the difference between mortalities in treatments and in controls was greatest for the newly manufactured materials. The results obtained in these tests show significantly lower mortalities than those reported in laboratory tests with American strains of lice (21, 22).

Bushland et al (22) reported that after lice had been exposed to 10% DDT powder for 3½ hours, no lice were capable of feeding, although only 60% of the lice had suffered "knock-down" by the insecticide at this time. They point out that the interference in feeding habits is of considerable significance since it would effectively prevent transmission of typhus, pending the lethal action of the insecticide. Observation on the Tokyo strains of lice indicate that DDT is ineffective in interfering with the feeding habits of these strains. Table XXVII indicates the observations made on the feeding habits of lice surviving exposure to DDT. After six hours of exposure, an average of 90% of the surviving lice fed readily, while an average of 80% still fed readily after 32 hours exposure.

Particle size of DDT dusts showed no correlation with mortality in lice, and did not exhibit any effect on the feeding habits of exposed lice.

A series of 18 tests was performed on lice, using six insecticidal preparations other than DDT to give a basis for comparison. Three tests were run on each compound. The techniques used were identical to those used in the pill-box feeding series of tests with DDT. The six compounds tested were as follows:

Compound #1 - 5% benzene hexachloride (1.5% gamma isomer) in talc.

Compound #2 - 0.2% pyrethrins, 2% piperonyl butoxide, 0.25% phenol S in talc.

Compound #3 - 0.22% pyrethrins in talc.

Compound #4 - 0.15% pyrethrins, 0.15% N-octyl bicycloheptene dicarboximid in talc.

Compound #5 - 0.46% allethrin in talc.

Compound #6 - 0.2% allethrin, 0.2% N-octyl bicycloheptene dicarboximid in talc.

Results obtained with these compounds are given in Table XXVIII. It will be noted that all of the compounds produced higher mortalities than DDT and that several gave complete kill within 24 hours.

The data presented herein establish that strains of human body lice, obtained from vagrants in Tokyo, are not completely controlled by DDT. Chemical analysis has indicated that several of the samples of DDT used in these tests were well within the specifications set for this insecticide. This study also establishes that these lice are highly susceptible to other insecticides, including benzene hexachloride, pyrethrum and allethrin. It must therefore be concluded that the strains of lice used in this work show a distinct resistance to DDT. Whether or not this resistance is natural or acquired cannot presently be demonstrated. However, it is believed that the lice used have not been previously exposed to DDT, at least not for several months.

SURVEYS FOR MALARIA AND SCRUB TYPHUS VECTORS IN FORMOSA AND THE PESCADORES ISLANDS: At the request of Headquarters, MAAG, Formosa, studies were undertaken on malaria and scrub typhus vectors on Formosa and the Pescadores Islands. One officer visited Formosa during the period 24 August - 4 September, and the same officer returned again with two enlisted technicians for a 7 week study during October and November. Studies were conducted in the Taipei - Keelung area in the north, along the entire length of the western coastal plains, in mountainous areas of the south and south east, and in

Table XXV. Results of 24-Hour Exposure of Lice to Five Year Old DDT

Sample No.	No. Tests	Mortality in Treatments No.	%	Mortality in Controls No.	%
3	3	15/30	50	6/30	20
4	6	36/60	60	6/30	20
5	9	58/130	45	27/110	25
	18	109/220	50	39/170	23

Table XXVI. Results of Exposure of Lice to DDT for 48 Hours

Test Series	No. Tests	24-Hr. Mortality Treatments No.	%	Controls No.	%	48-Hr. Mortality Treatments No.	%	Controls No.	%
"Old American"	20	228/400	57	86/360	24	297/400	74	164/360	46
"New American"	20	220/400	55	63/357	18	298/400	75	118/357	33
"New Japanese"	12	83/240	35	40/240	17	113/240	47	70/240	29
	52	531/1040	51	189/957	20	708/1040	68	352/957	37

Table XXVII. Effects of DDT Exposure on Feeding Habits of Lice

Test Series	6-Hr. Exposure No. Surviving	No. Feeding	% Feeding	32-Hr. Exposure No. Surviving	No. Feeding	% Feeding
"Old American"	369	312	85	172	135	78
"New American"	306	281	92	180	126	70
"New Japanese"	211	204	97	157	144	92
	886	797	90	509	405	80

Table XXVIII. Results of Insecticide Tests on Human Body Lice

Compound	24-Hr. Mortality Treatments No.	%	Controls No.	%	48-Hr. Mortality Treatments No.	%	Controls No.	%
#1	60/60	100	11/40	28	60/60	100	12/40	30
#2	60/60	100	11/40	28	60/60	100	12/40	30
#3	60/60	100	1/40	3	60/60	100	7/40	18
#4	56/60	93	1/40	3	60/60	100	7/40	18
#5	53/60	88	1/40	3	60/60	100	7/40	18
#6	40/60	67	1/40	3	50/60	83	7/40	18

the three main islands of the Pescadores group. Representing the Chinese Nationalist
Forces, and working with this survey team, was Major General T. L. Chang of the Nat-
ional Defense Medical Center. Taipei.

Malaria is the most important of the insect-borne diseases on Formosa (32, 33)
and, therefore, most of the activities of this team were devoted to the vectors of

this disease. The disease is most prevalent in the southern and eastern sections of the island. In the foothill regions infection rates of 20% to 35% have been found among the aborigines, while incidence is reported to range between 6% and 15% in the plains areas. It is questionable, however, if any substantial portion of the malaria reported from the plains areas is actually contracted in these areas. Formosa is widely covered with mountains, and plains are restricted to narrow coastal strips. While the greatest part of the population is concentrated in these coastal belts, foothills are always nearby, and many of these people undoubtedly are exposed to malaria in the foothill areas. In the northern section of the island almost all malaria is of the tertian type, while in the southern part of the island the incidence of tertian and subtertian (aestivo - autumnal) malarias are almost equal. Quartan malaria is rare throughout the island.

Sixteen species of anopheline mosquitoes have been reported from Formosa (34). All but two of these species were collected during the course of this survey, in which several thousand mosquito adults and several thousand mosquito larvae were collected. Several of these species require meticulous conditions for their breeding and consequently have a very limited distribution throughout Formosa. Other species such as Anopheles hyrcanus sinensis are common and widely distributed over the island. The most important vector of malaria on the island apparently is Anopheles minimus (35). This species breeds in vegetation on the edges of small strains in the foothill areas. A second species which has been considered of lesser importance in the transmission of malaria is Anopheles hyrcanus sinensis (35) which breeds most commonly in rice fields. There is some question as to whether or not this species actually transmits malaria on Taiwan (36). There is also a lack of information on the importance of several other species in the transmission of malaria.

Anopheles hyrcanus sinensis was found to be very common and was collected in almost every locality visited. Anopheles minimus was only taken in the foothill areas at such places as Chia tsu and Sintse (both near Chao Chow), Tama and Szeshan (near Taitung), and Keelung. It was never taken in the vicinity of any of the MAAG hostels. All of the MAAG hostels are in towns or cities lying well within the coastal plains, and since such towns and cities appear to have little indigenous malaria, it is believed that personnel whose activities are restricted to these areas have a rather limited exposure, if any, to malaria. Personnel working or travelling extensively in foothill country will be exposed almost constantly to malaria. Anopheline breeding is continuous throughout the year over the entire island.

The other insect-borne diseases offer much less danger than malaria. No information is available on the occurrence of murine typhus, while filariasis is reported to be very uncommon on the island (37). However, with the influx of Chinese troops from the mainland, filariasis may well have been introduced into the island, since earlier surveys (36) showed that 8% of the Chinese forces on the mainland were infected with this disease. During the Japanese occupation of Formosa, several outbreaks of dengue were reported (38, 39). The so-called "Taiwan fever" may be dengue, although it may also include cases of murine typhus. Aedes albopictus which is the most important vector of dengue on the China mainland, is very common and widely distributed throughout Formosa. Japanese investigators on Formosa were of the opinion that Armigeres subalbatus, which is very common on the island, was also implicated in the transmission of the disease (40).

Scrub typhus (tsutsugamushi fever) has been reported from the east coast of Formosa between Hualien and Taitung (41, 42) but the disease appears to be quiescent at present in these areas. Scrub typhus was currently occurring in the town of Tung-shih, which lies approximately 20 miles north-east of Taitsung on the western side of the island. This was confirmed by examination of patients and finding of typical eschars. During the past four years incidence of the disease has varied between 7 and 43 cases with case fatality rates up to 47%.

Scrub typhus was found to present the greatest problem of the arthropod-borne diseases in the Pescadores Islands (43) while malaria constitutes a minor problem. There are no accurate statistics on the actual incidence of the disease, but from information gathered from civilian and military physicians it appears that the Pescadores have an unusually high incidence of the disease. One civilian physician stated that he had treated approximately 150 scrub typhus patients during 1951. Statistics gathered from military physicians for the 3 year period 1949-1951 indicate the greatest incidence of the disease in the Chinese Nationalist Forces in the Pescadores to be in July and August. On Gyooto Island (Shihyu), the disease has been very prevalent, as evidenced by the fact that in 1949 7.9% of the troops on the island contracted the disease, while in 1950 and 1951 8.3% and 6.9% of the troops came down with the disease. Fortunately, the disease is extremely mild in comparison with that seen in other endemic areas and the mortality is extremely low. During the course of these studies, 47 rodents were trapped on the Pescadores Islands including 17 shrews, Suncus murinus swinhoe (Blyth), 27 field mice, Mus musculus formosanus Kuroda, and 3 rats, Rattus rattus rufescens (Gray). The chigger, Trombicula akamushi* was taken on all three species of rodents as follows:

Rodent Host	No. Collected	No. Positive	% Positive
Shrew	17	13	77
Field mouse	27	8	30
Rat	3	2	66

Although a larger number of shrews were found infested with T. akamushi than the other rodents, individual infestations on shrews were very light. The heaviest individual chigger infestations were found on the rat, Rattus rattus rufescens. Rats were trapped in or near houses, while the field mice and shrews were taken along rock fence rows or in groves of the shrub, Leucaena glauca. (Linn.). Rock fence rows are very common all over the islands, and a grass, Saccharum spontaneum indicum genuinum Hack., was commonly found growing along them. Abaca plants were also found frequently growing on or along these rock fences. None of the rodents referred to above can be considered "wild" rodents since all are commonly seen in or about human habitations. In fact, scrub typhus in the Pescadores Islands differs markedly from the classical epidemiological picture in that the disease is apparently frequently contracted in houses. Evidence of this is seen in the not infrequent occurrence of the disease among infants who have never left the home.

ORGANIZATION OF A TAXONOMIC ENTOMOLOGY SECTION IN KYOTO: A taxonomic entomology section of the Entomology Department was designated in September 1951 to function at Kyoto, Japan. The project is similar to projects conducted under the supervision of Lt. Colonel Walter J. LaCasse, USAF, and concerned with taxonomic studies of mosquitoes of various regions. Studies on the taxonomy of the mosquitoes by Colonel LaCasse and associates were officially terminated as of 31 August 1951. This action made available for use on other projects a group of seven highly skilled scientific illustrators, all Japanese Nationals residing in Kyoto. It was deemed advisable to utilize the talents of these scientific illustrators in the preparation of much needed publications dealing with other groups of medically important arthropods in the Far East.

The work being done at Kyoto is basically a part of a much larger proposed project involving a critical study of the mites (Acarina), fleas (Siphonaptera) and other medically important arthropods of the Far East. It is proposed that the project at Kyoto be concerned primarily with definitive taxonomic studies of mites and

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* Trombicula identifications were made by Dr. K. Asanuma, Research Institute for Natural Resources, Tokyo and by Dr. Y. Obata, Kitasato Institute, Tokyo.

fleas, and the preparation of the scientific illustrations necessary to make this information of practical value to units concerned with the investigation and control of human disease known or thought to be transmitted by these parasites. The mission of the project at Kyoto may therefore be defined as follows:

1. Immediate objective: The preparation of illustrated synoptic guides to the common mites and fleas of Japan and Korea, for the use of Medical Service units in the field.

2. Long term objective: The preparation of comprehensive and adequately illustrated publications dealing with the same groups. Through cooperative arrangements with other Medical Service units and scientific collaborators, these publications will be enlarged to contain all available pertinent biologic information.

Administrative and technical supervision is by the 406th Medical General Laboratory. Logistical support, to date, has been primarily by the U. S. Army Hospital Kyoto, 8164 Army Unit. This support involves laboratory space, the major portion of supplies and equipment, and the hire of Japanese personnel. Personnel now engaged in this project at Kyoto include one officer in charge, one artist supervisor, six artists and one slide technician. One civilian specialist (DAC) will shortly join this group.

Illustrations of some 19 species or stages of mites from Japan, Korea, and North America have been completed or are nearly complete. Preparation of slide mounts of collected material from Japan and Korea is being accomplished as rapidly as facilities and personnel permit. Reference collections of mites for the use of Medical Service units are being assembled as slide mounted material becomes available.

FIELD SURVEYS OF RODENTS AND RODENT ECTOPARASITES: During the latter part of 1951 an organized program for the study of rodents and rodent ectoparasites was undertaken. Objectives of this program were: (a) to gather information on the ecology, biology and taxonomy of rodents and rodent ectoparasites in Japan and other areas of the Far East Command, (b) to integrate this information with projected studies on the epidemiology of Epidemic Hemorrhagic Fever, (c) to determine if rodents or rodent ectoparasites play a role in the epidemiology of Japanese B encephalitis, and (d) to determine what role these rodents and rodent ectoparasites may play in the epidemiology of leptospirosis in Japan. Late in the year one officer and two enlisted technicians were assigned to this program. As the year ended field studies at the Urawa and Chichibu areas of Saitama prefecture in Japan were begun.

The Department of Virus and Rickettsial Diseases for the past four years has been responsible for laboratory confirmation of infectious diseases due to filtrable agents occurring in Occupation and United Nations troops in this theater. The Department has also provided laboratory facilities and technical personnel necessary for the compilation of epidemiological data relevant to the study and control of these diseases.

In the previous years during which this department functioned, Japanese B encephalitis and scrub typhus were the two diseases, in this general group, which had the highest incidence and which therefore commanded the most attention. The past year, however, saw little of either of these diseases among United Nations troops. Efforts were continued to extend certain of the basic observations made in the past concerning segments of the natural history of Japanese B encephalitis but these efforts fell short of their goal. Several projects whose evaluation and completion depended upon a rather widespread dissemination of this virus in nature suffered materially by the absence of the local occurrence of Japanese B encephalitis in a significant degree.

For the first time since 1945 smallpox appeared in epidemic proportions in United Nations forces in the Far East. This disease, together with an entity unfamiliar to Western medicine, "epidemic hemorrhagic fever" provided much of the workload of 1951.

The status of the diagnostic routine, together with a summary description of some of the special projects with which this department has been concerned, provides the material for the following report.

ROUTINE EXAMINATIONS: The following tabular summaries indicate the extent both in type and in number of the tests performed (Tables I and II).

Japanese B Encephalitis - In contradistinction to the experience with epidemic encephalitis in the Orient in 1950, little Japanese B encephalitis was encountered in the United Nations Forces in the summer and fall of 1951. A total of 68 cases was reported as having occurred in United Nations personnel in this theater in 1951. This figure is taken from records compiled by a radio reporting system in which all cases of central nervous system infections are reported, together with the initial clinical diagnosis as soon as hospitalization was effected. In many instances, the clinical impression was changed later in the course of the illness.

Similarly, Japanese physicians encountered fewer cases in the native population this year as compared with the preceding year. The Japanese Ministry of Health reported 2,139 cases of suspected encephalitis in 1951. This compares with 5,196 reported cases in 1950.

Serum for serological confirmation was received from 428 patients upon whom the diagnosis of Japanese B encephalitis was being considered. This included cases occurring in native Okinawans as well as those in United Nations troops in Korea and American forces in Japan and Okinawa. Submission of an inadequate number of serum samples made definitive serological studies impossible in 285 of these cases. Most of the suspected cases upon whom inadequate numbers of serum samples were submitted were native Okinawans.

In the remaining 143 patients, upon whom adequate serum samples were available, serological evidence of recent exposure to Japanese B encephalitis virus was obtained in ten patients. The criterion for serological confirmation by the complement fixation tests during the past year was identical to that described in the 1950 Annual Report of this laboratory (1), and for a definite diagnosis demanded either a four-fold

Table I. Serological Procedures - 1951

| | SPECIFIC TESTS PERFORMED | | | | | | | | | |
| Type of Serological Test | Neurotropic Viruses | | | | Typhus | | Pneumotropic Agents | | | Misc. |
	JBE	SLE	WEE	EEE	Epi.	Mur.	Q-Fever	Psitt-LV	Influenza	Variola-Vaccinia
Complement Fixation	2913	213	141	19	93	93	51	54	--	58
Neutralization	2880	20	6	--	--	--	--	--	--	--
Rickettsial Agglutination	--	--	--	--	84	84	--	--	--	--
Hemagglutination Inhibition	1304	--	--	--	--	--	--	--	310	3

Table II. Isolation Attempts - 1951

| | Tissue | | | | | |
Type Infection	Brain	Spinal Fluid	Blood	Throat Washings	Other	Total
Central Nervous System - JBE	23	4	3	--	163°	193
Rabies	49	--	--	--	--	49
Respiratory Influenza	--	--	--	49	--	49
Atypical Pneumonia	--	--	20	25	--	45
Newcastle Disease	--	--	--	--	24ƒ	24ƒ
Dermatotropic Smallpox	--	1	8	--	24°° 2,000°°°	35
Epidemic Hemorrhagic Fever	--	--	18	1	3* 12** 1***	35

° Mosquito Pools
°° Vesicular Fluid, Skin
°°° Kidney, Spleen
* Blood, Bone Marrow
** Liver, Kidney and Spleen
*** Mites and Lice
ƒ Chicken Tissue

rise or a sustained titer of 1:16 in complement fixing antibody when acute and convalescent phase sera were compared. Neutralization tests supported the positive complement fixation tests in the nine cases from whom sufficient serum for testing was submitted, one patient having insufficient serum for the neutralization test (Table III).

Table III. Serological Evidence of Japanese B Encephalitis - 1951

Patient	Presumed Location of Exposure	Lab. No.	Day of Disease	Complement Fixation	Neutralization Index
W. M.	Okinawa	V1-2887	6	0	2,000
		V1-4488	59	1:32	NT*
R. M.	Okinawa	V1-3752	6	1:8	>5,000
		V1-4439	44	1:32	>5,000
W. W.	Okinawa	V1-3753	6	0	> 320
		V1-4278	35	1:8	>2,200
R. N.	Japan	V1-4608	8	1:8	>4,000
		V1-4703	25	1:128	800
E. P.	Korea	V1-4549	5	1:4	>3,200
		V1-4563	12	1:8	2,500
		V1-4570	15	1:8	>3,200
		V1-4611	18	1:8	3,200
		V1-4846	46	1:16	NT*
S. J.**	Okinawa	V1-4071	7	0	16
		V1-4470	21	1:8	200
A. E.**	Okinawa	V1-3759	8	0	>4,000
		V1-4068	22	1:8	400
K. N.**	Okinawa	V1-3763	7	0	QNS***
		V1-4072	21	1:8	QNS***
T. N.**	Okinawa	V1-3756	13	1:32	2,500
		V1-3841	20	1:32	> 2,500
Y. V.**	Okinawa	V1-4060	3	0	NT*
		V1-4468	22	1:8	>6,300
		V1-4471	33	0	>6,300

* Not Tested
** Native Okinawans
*** Qauntity Not Sufficient

Effort was made to recover an infectious agent from 30 specimens received in the laboratory from patients with central nervous system infection. These included 23 necropsy specimens of brain, four spinal fluids and three whole blood samples. Two strains of neurotropic viruses were recovered, both from human brain, and presumptively identified by complement fixation with, and neutralization by, known hyperimmune animal sera, (Table IV).

A total of 12 cases of central nervous system infection were shown to be caused by Japanese B encephalitis virus. Ten cases were proven serologically: five Americans and five Okinawans. Two cases in Americans were shown to have been, by isolation of the virus, the result of Japanese B encephalitis.

Table IV. Isolation of Japanese B Encephalitis Virus From Humans - 1951

Lab. No.	Case	Presumed Location of Exposure	Date-Onset	Date/Death
Vl-4562	R. P.	Korea	9-7-51	9-13-51
Vl-4993	D. B.	Okinawa	11-6-51	11-15-51

Rabies - Rabies virus was recovered from six of 49 animal brains submitted to this laboratory in 1951. All six isolations were made from dog brains. Negri bodies were seen in but five of the 49 original specimens, and the presence of rabies virus was confirmed by mouse inoculation in each instance in which Negri bodies were observed.

Typhus - Serum from a total of 189 patients suspected of having typhus was received, with only 53 cases of the total number having serum sampling adequate for laboratory studies. The Weil-Felix reaction and the complement fixation test were used in the initial serological screening. All sera fixing complement with washed rickettsial antigen were evaluated by rickettsial agglutination. There was no serologically confirmed typhus in the United Nations forces in 1951. No material was received for attempts at rickettsial isolations.

Scrub Typhus - No scrub typhus was reported in Allied troops stationed in Japan during the past year. However, three possible cases occurred almost simultaneously in members of the Australian Battalion assigned to the British Commonwealth Division in Korea during the month of June. All three cases were reported as having the typical clinical picture of scrub typhus, and as having responded to chloramphenicol treatment. There were no efforts made to recover R. orientalis. The Weil Felix reaction with Proteus OX-K rose diagnostically in all three patients, during the course of the illness.

Influenza - Table V summarizes the serological experience with influenza in 1951.

Table V. Serological Experience With Influenza - 1951

Period	Paired Sera Tested		Distribution of Diagnostic Rise			
	Total	Diagnostic Titer Rise	PR-8	FM-1	Lee	Multiple
Jan - April	117	32	18	26	3	13
May - Aug	24	0	0	0	0	0
Aug - Dec	4	0	0	0	0	0
Total	145	32	18	26	3	13

No transmissable hemagglutinating agent was recovered in 49 isolation attempts made January through April 1951, inoculating antibiotic-treated throat washings into 10-day embryonated eggs.

The total number of paired serum samples received for influenza antibody assay reflects the common respiratory disease rate for the Far East Command fairly accurately. Most diagnostic titer rises occurred to the A group of influenza viruses (Table V) and all titer rises were demonstrated during the first three months of the year. It is apparent that influenza occurred in this theater simultaneously with the epidemics that were seen in Europe and in the United States during the first few months of 1951. Unfortunately, no virus was recovered from patients in this theater so that strain comparison cannot be made with American and European agents.

Atypical Pneumonia - Complement fixation tests for Q-fever and Psitt-Lymphogranuloma Venereum type infections were done throughout the year on serum samples from sporadic cases of atypical pneumonia. In no instance was complement fixing antibody

demonstrated. Opportunity to study a circumscribed outbreak of pulmonary disease presented itself in the 8th Station Hospital in Kobe, Japan, during March 1951. Results of this study will be found in the report of the Epidemiology Section.

Variola - A detailed description of the study of the cases of smallpox which occurred in Korea during the first six months of 1951 will be found in the Epidemiology Section of this report. Specimens for isolation attempts were received on 35 patients upon whom the clinical diagnosis of smallpox was being considered. These specimens yielded isolates from 11 patients. Tables VI and X summarize briefly some of the pertinent results of these isolation attempts.

Table VI. Distribution of Eleven Virus Isolates Variola-Vaccinia Group 1951

Source of Isolates	Source of Specimens	Month	Received
9 - from vesicle fluid	6 - from 3rd Sta Hosp	January	2
	2 - from 8th Sta Hosp	February	2
1 - from skin and kidney	1 - from 395th Sta Hosp	March	3
	1 - from 155th Sta Hosp	April	2
1 - from serum	1 - from US Nav Hosp	May	1
		June	1

Biologicals - The following table summarizes the production, by this department, of diagnostic reagents made in the last year.

Table VII. Biological Production - 1951

	Antigens (ml)	Hyperimmune Serum (ml)
Normal Mouse Brain	430	-
Japanese B Encephalitis	1,015	163
St. Louis Encephalitis	551	35
Eastern Equine Encephalomyelitis	222	16
Western Equine Encephalomyelitis	120	24
Influenza PR-8	390	62
Influenza FM-1	465	42
Influenza Lee	180	35
Vaccinia	15	69
Total	3,388	446

SPECIAL PROBLEMS: The following descriptions, which of necessity have been kept rather brief, summarize the major research efforts made by this department in the past 12 months. The direction of these research efforts was dictated largely by the experience of previous years, as in the case with Japanese B encephalitis and influenza. The necessity to cope with specific problems in infectious diseases as they arose in this theater provided the background for the studies of variola, atypical pneumonia, and epidemic hemorrhagic fever which are described in the following pages.

The Effect of Frozen Storage Upon The Ability To Recover Japanese B Encephalitis Virus From Mosquitoes - A large number of mosquitoes collected, identified, and tubed for virus isolation attempts during the 1950 Japanese B encephalitis season made possible a study of the virus recovery rates, comparing freshly pooled mosquitoes with those which had been frozen for periods of time ranging up to eight months. In 1950, in so far as it was possible, isolations were attempted immediately after the mosquitoes were collected, killed, identified, and tubed. When the volume of mosquitoes collected on any given day exceeded that capacity which could be properly processed, they were sealed in glass ampules in lots of 100 and stored at -50°C until such time as they could be processed. The frequency with which virus could be recovered from these frozen lots was compared with that of fresh mosquitoes.

Materials and Methods - Mosquito collection and identification were made by the Department of Entomology. Tubed mosquitoes pooled by species were turned over to this department for isolation attempts. The techniques used for these attempts were identical to those described in the 1950 Annual Report of this laboratory (1).

        Results - Of the 61 pools of C. tritaeniorhynchus collected between 3 July and 8 September 1950 and inoculated from the fresh state, 13 serologically confirmed isolates were recovered, a rate of 21 percent. Using lots of mosquitoes collected over the same period of time, but tested after storage in the frozen state for eight weeks, 25 of 133 lots (19 percent) contained Japanese B encephalitis virus. Only 15 of 147 lots (10 percent) were positive for virus when tested after 7-8 months of frozen storage. Superficially, it would appear that prolonged storage at -50°C reduced the recovery rate approximately 50 percent.

        However, when these three groups are broken down by week of collection (Table VIII) the data suggests that groups represent heterogeneous mosquito populations. Statistical comparison of virus isolation rates made from such populations cannot be conclusive. It only suggests that frozen storage of naturally infected mosquitoes for eight months may possibly reduce the recovery rate of Japanese B encephalitis virus.

        It is interesting to note that a majority of isolates were recovered from collections made during the 12 day period 31 July to 11 August 1950. This time period immediately precedes the peak week of 13 August for the onset of Japanese B encephalitis in patients and follows by two weeks the peak C. tritaeniorhynchus population. These observations, indirectly, give added support to the hypothesis that C. tritaeniorhynchus may serve as a natural vector of Japanese B encephalitis in humans and does not serve as the natural reservoir of infection.

        Japanese B Encephalitis Virus in Mosquitoes - 1951 - Extensive experience gained during the summer of 1950 indicated that Japanese B encephalitis virus could be recovered from wild caught C. tritaeniorhynchus mosquitoes but that virus was present in mosquitoes for only a short period of time. During the past season an attempt was made to extend these observations. That part of isolation studies conducted by this Department is presented herewith. A more complete summation is included in the report of the Entomology Department.

        Isolations during the summer of 1951 were attempted from only two mosquito species, C. tritaeniorhynchus and A. hyrcanus sinensis. Previous experience had found C. tritaeniorhynchus to be naturally infected with Japanese B encephalitis virus but both the anothelene and the culecine mosquitoes have been reported by Japanese investigators as being naturally infected.

        Mosquitoes were trapped in the Tokyo metropolitan area by the Department of Entomology using animal bait traps, light traps, and resting stations. Mosquitoes were killed, identified, tubed by species, and turned over to this department for further processing.

        Insofar as possible, mosquito lots were processed and inoculated from the fresh state. When this method was not practical, pools averaging 35 mosquitoes were glass-sealed in ampules and stored at -50°C until time permitted processing. In no instance was this storage period more than nine days.

        An entire lot of mosquitoes was suspended in phosphate buffered saline (pH 8.2) containing 1000 units of penicillin and 500 micrograms of streptomycin per cc. After emulsion in a Ten Broeck grinder the mosquito suspension was held at 4°C for at least one hour to allow the antibiotics to suppress bacterial contaminants. Each suspension was then centrifuged and the supernatant used to inoculate five 8-10 gm albino mice (0.03 cc intracerebrally and 0.1 cc intraperitoneally). The mice were observed twice daily for 14 days, and any animals showing central nervous system symptoms were sacrificed and the brains harvested. Such brains were sub-passaged to groups of ten mice each. If typical symptoms occurred in the second passage and could be transmitted through four serial passages a complement fixing antigen was prepared from the infected brain. The antigens were then tested for their capacity to fix complement in the

Table VIII. Mosquito Isolation, 1950 - by Week

Mosquito Lots Tested and Isolates Obtained by Week

		3 July	10 July	17 July	24 July	31 July	6 Aug	13 Aug	20 Aug	27 Aug	3 Sept	Totals	Overall % Recovery
Fresh	Attempts	8	7	2	12	16	7	2	1	4	2	61	
	Isolates	0	2	0	2	5	3	0	0	1	0	13	21%
Frozen 8 weeks	Attempts	11	26	20	14	10	18	18	12	3	1	133	
	Isolates	0	1	2	3	1	10	8	0	0	0	25	19%
Frozen 8 months	Attempts	6	29	53	23	26	9	1	0	0	0	147	
	Isolates	0	0	2	5	7	1	0	0	0	0	15	10%

presence of Japanese B encephalitis, St. Louis encephalitis, or Western equine enceph-
alomyelitis immune serum. Specific fixation of complement by any antigen to a dilution
of 1:8 or better was considered presumptive evidence that the unknown agent was the
virus of Japanese B encephalitis.

A total of 163 lots of mosquitoes were inoculated. This included 134 of C. tri-
taeniorhynchus and 29 of A. hyrcanus sinensis.

Mosquitoes tested by this department were collected daily except on Sundays from
22 June 1951 to 5 September 1951. Unlike the previous year, the apparent mosquito pop-
ulation was small and the sparse number of mosquitoes was reflected both in the number
of pools collected and in the size of the pools individually. From a total of 134 lots
of C. tritaeniorhynchus mosquitoes, 11 lots were found to be naturally infected. None
of the anophelene mosquito lots yielded isolates. The lots yielding Japanese B enceph-
alitis virus were all collected between 17 August 1951 and 1 September 1951. Since
total collection was discontinued on 5 September 1951 no statement can be made concern-
ing the length of the period during which mosquitoes were found to contain virus; how-
ever, virus recoveries began at a definite date in mid August. It is interesting to
note that C. tritaeniorhynchus population became infected with Japanese B encephalitis
virus approximately one month later this year than the previous year. Suggested reasons
for this phenomenon will be found in the Entomology Section of this report.

Effort must be made in succeeding years to determine whether the appearance of Jap-
anese B encephalitis virus is as abrupt in time as it now appears to be. Further study
is needed to determine the actual incidence of mosquito infection by week, and how much
virus is actually present in infected mosquitoes.

Before any critical evaluation of the role of this mosquito species in the natural
history of Japanese B encephalitis can be made, it will be necessary to determine wheth-
er or not naturally infected mosquitoes are capable of transmitting the disease exper-
imentally, as well as the relationships between the virus content of the mosquito and
its capacity to induce infection.

Japanese B Encephalitis: Survey for Inapparent Infection, 1951 - Following the
1950 epidemic of Japanese B encephalitis in Korea and Japan, data was obtained by samp-
ling a group of soldiers which suggested that a high rate of inapparent infection ex-
isted (1). Approximately 50 percent of a nonvaccinated susceptible military population,
with exposure for a limited period were found to have significant levels of circulating
neutralizing antibody (NI = 1000 or greater) when bled within 1 month after exposure.
Since the total population tested was small, it seemed advisable to make the effort to
determine the relative inapparent infection rates in Korea and Japan during the 1951
season.

The present survey was begun early in the past summer, just prior to the administra-
tion to all troops in the Far East Command of either a full course of chick embryo vac-
cine or, depending upon a history of previous vaccination, a single booster injection.

Serum was obtained by serial bleeding of men from several Army units in Japan and
Korea during early summer and late fall of this year. The groups surveyed from the Jap-
anese islands were stationed in Tokyo and in Hokkaido. Samples of serum obtained from
these men before and after each vaccination and others taken in late fall, are available
on 50-100 men from each of these two groups. Men from three units stationed in Korea
supplied the serum for the remainder of this survey; they were bled only twice, after
vaccination in late fall. Serum from 50-100 men in each of these three groups, which
included troops from combat areas and from an isolated island, has been obtained. In
addition 400 fresh troops were bled on arrival in Japan for comparison with 400 being
rotated from Korea.

While there was little evidence of widespread dissemination of virus in nature during the past season, assay for antibody to Japanese B encephalitis in these serum specimens is now being made, and a final report will be rendered at a later date.

Passive Protection of Horses Against JBE Virus - Hyperimmune serum has been repeatedly employed in the past to alter or prevent specific viral diseases in both man and in experimental animals. That human gamma globulin can effectively modify measles when given in adequate amounts is well known by American clinicians. Susceptible animals can be effectively protected from experimental virus infections by the use of specific hyperimmune serum. Recently, Stokes (2) has used gamma globulin to provide a short period of passive immunity in human volunteers to infectious hepatitis virus, and has shown that such protection was enough to allow the subsequent challenge of such individuals with living virus to produce an asymptomatic infection with ultimate reinforcement of the immunity. It seemed reasonable in the light of these observations to determine what effect the administration of gamma globulin prepared from Japanese plasma would have upon natural infection of experimental animals.

Horses were chosen as the most appropriate experimental animals since it had been shown (1) that the horse readily developed neutralizing antibody to Japanese B encephalitis virus when exposed during but one epidemic season. Gamma globulin was obtained from both American (plasma fraction) and Japanese (placental fraction) sources. It was planned to give these fractions to the horses shortly before a time, based upon previous years' observations, when mosquitoes could be shown to be carrying Japanese B encephalitis virus.

A standard American preparation of gamma globulin (Squibb) was tested for the presence of neutralizing antibody for Japanese B encephalitis virus (Nakayama strain) by the intracerebral and intraperitoneal techniques in albino mice. Serial ten-fold dilutions of the virus were added in series to each of three different dilutions of gamma globulin (160 mgm, 80 mgm, and 40 mgm per cc) using normal rabbit serum as the control, and incubated two hours at 37°C. For each of the three tests and the control in the intracerebral titration, 0.03 cc of each mixture of virus and globulin was inoculated and 0.1 cc was given in the intraperitoneal titration. Neutralization indices obtained by either techniques were less than 10.

A trial pool of approximately 20 cc of gamma globulin obtained from Japanese sources was tested as outlined above. The neutralization index for this preparation was 400. The gamma globulin obtained from the Japanese was placental extract with a protein concentration of 5.2 percent of which only 62% was gamma globulin.

Since it was considered doubtful that a gamma globulin product containing neutralizing antibody levels of only 400 would be of any value when administered to experimental animals it was decided to use high titered whole Japanese plasma in the following experiment. Two Hokkaido horses one to two years of age, which were serologically negative to Japanese B encephalitis virus were selected along with appropriate control horses to determine what degree of passive immunity could be given them. Horses #1 and #2 were given intravenously 725 cc of immune pooled Japanese plasma with a mean neutralization index of 7,400. Control horses #3 and #4 were each given 725 cc of saline while horses #5 and #6 each received intramuscular injections of 50 cc of American gamma globulin. This quantity of gamma globulin is approximately equivalent to the amount of gamma globulin contained in the Japanese plasma given to the two test animals #1 and #2. All animals were bled prior to and immediately after injection, and bled thereafter on seven consecutive days. Subsequently bi-weekly bleedings were made for a period of one month. Table IX summarizes the results of the experiment.

Intravenous administration of Japanese B encephalitis virus antibody to horses allows the immediate demonstration of circulating neutralizing antibody. These antibodies are maintained well for about seven days. Between the 7th and 21st day a gradual fall in antibody was demonstrated. In none of the control animals could any antibody be demonstrated during simultaneous testing periods. It is interesting to note that by the 51st day of the experiment five of the six horses had developed neutralizing antibody which persisted for at least two months. This presumably can be attributed to natural infection.

Table IX. Neutralization Index Logs In Horses Passively Immunized To Japanese B Encephalitis Virus

Horse No.	Before Injection	After Injection	Day												
		0	1	3	5	7	16	21	23	31	27	44	51	61
1	0	3.1	>3.3	3.2	2.7	2.8	1.1	0.8	3	>3.7	>4	>3.6	>3.6	>4.1
2	0.5	2.3	2.7	2.6	2.4	2.6	2	0.9	2	2.1	2.9	>3	3.1	>4.1

Given 725 cc Pooled Japanese Plasma (NI - 7400) Intravenously

Horse No.	Before Injection	After Injection	Day												
3	0.6		0.2	1	0.7	0.7	NT	0.9	0.9	<0.7	1	>3	>3.6	>4.1
4	0.8		0.8	1.1	0.8	0.6	1.1	1.8	<0.9	<0.7	1.1	>0.6	1.2	<1.1

Given 725 cc Saline Intravenously

Horse No.	Before Injection	After Injection	Day												
5	1.1		1.2	1	0.7	0.7	0.4	0.7	QNS	<0.7	2.1	2.8	>3.6	>4.1
6	0.8		0.9	2.9	1.4	0.8	NT	0.8	<0.9	<0.7	3.7	>3	>3.6	>4.1

Given 50 cc American Gamma Globulin (Squibb) Intramuscularly

It is obvious that the passive protection afforded these two horses did not prevent the natural infection which was manifested by the appearance of persisting neutralizing antibody. Whether or not such inapparent infection could be suppressed by the unbroken maintenance of artificial antibody levels is a question still to be answered.

There is no evidence that American gamma globulin can produce passive immunity to Japanese B encephalitis virus either by contributing neutralizing antibody or other unknown effect.

Antibody Response to Standard Influenza Vaccine - Incident to institution of an influenzal vaccination program an attempt was made to determine the effect of vaccination with standard polyvalent influenza vaccine upon hemagglutination-inhibiting antibody for influenza virus in humans. Both military and civilian personnel assigned to the 406th Medical General Laboratory were used in this study. Of 141 participating individuals, all were bled prior to immunization, and 73 were inoculated with 1 cc of standard influenza vaccine 8-9 days after initial bleeding. The entire group was bled again 15 days after vaccination. The paired serum specimens were tested by the standard hemagglutination inhibition technique against PR-8, FM-1 and Lee antigens. Of the 73 vaccinated individuals, 33 (45.2%) of them demonstrated a 2-tube rise against one or more of the 3 test antigens. Of the 68 persons who received no vaccine, only one demonstrated a titer rise during the same period. A complete summary of the serological responses to vaccine is shown in Table X.

Table X. Antibody Response to 1 cc of Polyvalent Influenza Vaccine

Group	Multiple Response			Single Response			Paired Sera Tested	Serum Response
	PR-8 FM-1	PR-8 Lee	PR-8 FM-1 Lee	PR-8	FM-1	Lee		
Vaccinated	2	7	4	5	1	14	73	33(45.2%)
Nonvaccinated	0	0	0	0	0	1	68	1(1.5%)

Epidemic Hemorrhagic Fever - The appearance of a disease entity characterized by fever, a tendency to hemorrhage, generalized toxicity, and varying degrees of renal damage in troops of the United Nations Command in Korea was noted early in July of this year. Since that time this disease, which was known to Japanese medicine since 1939, has been studied by Armed Forces clinicians, epidemiologists and pathologists. Pertinent clinical, pathological, and epidemiological descriptions of this disease will be found in the Epidemiology and Pathology sections of this report.

Early in July, at a time when the diagnosis of leptospirosis was being entertained for those patients who exhibited a febrile hemorrhagic diathesis, attempts were made by both field and fixed laboratories to recover leptospira from patients' blood and urine. Standard accepted techniques for the recovery of leptospira, when applied in a reasonable number of instances, failed to produce the consistent experimental illness in inoculated animals which would have occurred had the disease in question been of leptospiral origin. At the same time that various laboratories were failing to recover this organism, increasing clinical experience suggested that the disease in question was different from any entity that Western medicine had seen. Accordingly, attempts at the isolation of the etiologic agent were re-directed.

From the outset, it was apparent that the agent being sought was fastidious. No transmissable experimental disease could be established in albino mice, guinea-pigs, rabbits, or monkeys inoculated with tissue emulsions of liver, kidney and spleen

obtained at necropsy from eight different cases. Over an observation period of 30 days mice, rabbits, and monkeys inoculated intraperitoneally remained symptom-free. Febrile reactions in 350-450 gm guinea pigs occurred occasionally, usually between the 5th and the 14th day after inoculation, but such temperature rises were irregular both in time of appearance and in duration, and not all inoculated animals developed fever. Blood taken from such febrile animals at the peak of fever was inoculated intraperitoneally into additional guinea pigs. Similarly, liver, kidney, and spleen taken from sacrificed febrile guinea pigs was passaged to additional animals. Second animal passages were either nonproductive of specific symptomatology or ended in the death of the passage animal within 48 hours after inoculation. Penicillin and streptomycin were added to such contaminated inocula, and animals inoculated with such material failed to develop fever or specific symptoms during a 30-day observation period. Febrile animals, when sacrificed and autopsied, were not found to have any gross pathological changes in the major viscera; no definite histological evidence of local or systemic infection could be demonstrated.

The initial unrewarding attempts at recovery of the etiologic agent were in part a reduplication of the experience of Japanese investigators who had been unable to produce objective disease in laboratory animals inoculated with suspensions of human necropsy tissue. The opinion that the infectious agent would more likely be present in human blood or tissues for only a short period of time after the onset of fever was one that was shared by both this laboratory and by Ibuki and Kasahara (3) as well. Accordingly, whole blood was obtained from patients hospitalized in Korea with definite clinical evidence of the hemorrhagic form of this disease. Clotted blood was sent by air to this laboratory refrigerated in wet ice, arriving within 24-48 hours after it was drawn. Each of five specimens was inoculated intraperitoneally or subcutaneously after emulsifying the clot and serum in saline into guinea pigs, mice, suckling mice, rabbits, monkeys, and hamsters. The size of the inoculum each animal received was, of necessity, relatively small. No animal disease was observed during a one month observation period.

Early in September 1951 more complete clinical descriptions of epidemic hemorrhagic fever were obtained by this laboratory. These indicated that it was not until late in the first week of illness, or early in the second, that frank hemorrhagic phenomena appeared. Based upon knowledge of other diseases of virus or rickettsial origin, it seemed reasonable to assume that if the infectious agent were blood-borne at all, the peak circulating level would perhaps occur during the first or second day of disease. This idea was supported by observations made by Japanese investigators who reported that the agent could not be recovered from blood after the early febrile period of the disease. Since all blood used in previous isolation attempts had been obtained after the appearance of frank hemorrhagic disease, it was felt that these attempts should be repeated using more suitable material. This decision posed the immediate problem of determining the criteria for the selection of cases upon which isolation attempts were to be made. Appraisal of clinical records of such patients as were being evacuated to Japan at this time yielded little information as to the clinical manifestations of the disease in the first five days. Most of the patients were being seen by a series of medical officers in an evacuation chain, none of whom observed a patient long enough to clearly define his symptoms. Before a logical system for selection of cases for isolation attempts could be formulated it would be necessary to devise a practical method for predicting the eventual outcome of suggestive febrile illnesses. A representative of this laboratory, in cooperation with Medical Section, Eighth Army, was sent to Korea with the express purpose to define, if possible, a complex of symptoms and signs characteristic of the pre-hemorrhagic phase of this disease. This would obviate the necessity of indiscriminate bleeding of all fevers of undetermined origin and of holding such bloods until definite knowledge of the outcome of the suspected illness became available. Using such a symptom analysis it was found possible to predict with reasonable accuracy which patients would ultimately develop the typical picture of epidemic hemorrhagic fever. The sign-symptom complex of fever, headache, facial flush, periorbital edema and albuminuria was utilized as the standard for bleeding in the following experiments.

Thirty to 40 cc of blood, obtained within the first 72 hours after the onset of fever, was delivered to the laboratory either frozen or refrigerated in wet ice within 8-16 hours after bleeding. This blood was immediately inoculated according to the schedule shown in Table XI below. Each group of animals was inoculated with material from one patient, diluted with saline to sufficient volume (30 cc blood and 5-10 cc saline). The six best lots were chosen for this experiment.

Table XI. Inoculation Schedule of Isolation Attempts From Human Blood

Animal	I.P.	I.C.	S.Q.	I.V.	I.T.
Six -adult mice	X	X	-	-	-
Six -suckling mice	X	-	-	-	-
Two -hamsters	X	X	-	-	-
Two -guinea pigs	X	X	-	-	-
Two -rabbits	X	-	-	X	X
One -monkey	X	-	X	X	X

As in previous attempts, close observation of the inoculated animals which included twice daily temperature recordings on rabbits, guinea pigs and monkeys, daily white cell and differential counts on monkeys as well as daily inspection of inoculation sites where practical, failed to demonstrate concrete evidence of local or systemic disease. Significant febrile responses in the animals inoculated were conspicuous by their absence. Hematologic studies failed to reveal any significant blood changes in the monkeys.

Two rabbits, each inoculated with the same original starting material according to the schedule indicated in Table XI developed edema without necrosis in the inoculated testicle between the 5th and 7th day after inoculation. There was no concomitant evidence of systemic disease or of fever. The following pragraphs describe in detail the procedure followed with these two animals.

Rabbit No. 1 - This animal was operated on five days after inoculation. Under ether anesthesia the skin of the scrotum over the edematous testicle was incised and the parietal tunica with it. The wound gaped but the testicle did not deliver freely, being bound fairly well to the parietal tunica by adhesions. Blunt dissection finally delivered a testicle, dull red in color, approximately twice the normal size. The cut surface of the testicle bulged, but the capsule parted from the organ with ease. Sections of the diseased organ were fixed in formalin and the remainder of the testicle suspended in saline and passaged intratesticularly into two additional rabbits. No local lesion or systemic disease was observed in the inoculated testicles of the two passage rabbits. Histologically, the diseased testicle showed extensive edema with a minimal amount of mononuclear cell infiltration. There was no histological evidence of necrosis.

Rabbit No. 2 - This animal was operated on seven days after inoculation. Evidence of local swelling without necrosis was seen in the inoculated testicle on the 7th day after inoculation. Surgical removal of the diseased testicle showed it to be enlarged 1-1/2 times the normal size but not adherent to the parietal peritoneum. This testicle was treated similarly to the one described above, and inoculation failed to induce a similar lesion in passage animals. Pathologically, no significant lesion was noted in the original testicle.

Three similarly obtained acute blood specimens were pooled and inoculated intravenously into a horse. Observation of this animal over a 90-day period failed to demonstrate any evidence of systemic disease. Urine specimens and blood counts obtained from this animal every other day or twice weekly for the first 50 days failed to show any significant abnormality.

It seemed unlikely, in early October, that ordinary isolation techniques would be adequate and, although observations and passages of previously inoculated animals was

continued, more consideration was given to applying some of the more unusual techniques to the problem of isolation of the infectious agent. Previous to this time most blood for inoculation had been obtained in Korea and shipped to the 406th Medical General Laboratory. In order to minimize the possibility of inactivation of the infectious agent while in transit, a plan was devised which called for the inoculation of acute phase blood into animals at the bedside.

Accordingly, guinea pigs and mice were inoculated, at the bedside, with acute phase blood from selected patients. The criteria for selection of the patients was similar to that described previously, and four patients selected in the first 48 to 72 hours of illness were used in the following attempts.

In two instances, 5 cc of acute phase blood was inoculated into each of six mice by the intraperitoneal method. In one instance the inoculum consisted of an emulsion of sternal bone marrow in homologous whole blood. The other group of animals was inoculated with whole blood, and one of the guinea pigs was re-inoculated intraperitoneally on the same day with sternal marrow. These animals were returned immediately to the laboratory. The mice inoculated from these four patients were held for a 40-day period without observing the development of systemic disease. The guinea pigs from each of these series were sacrificed, and the organs passaged according to the following plan: - One guinea pig was sacrificed on day 5 or 6, one on day 9-11, and one on day 14-17 after inoculation. Tissue emulsions of liver, kidney, spleen, gonad, and mammary gland, if available, were passed intraperitoneally into three guinea pigs. Animals in the second generation were subjected to similar sacrificial schedules, and blind passage by this method was continued through five generations without definite indication of the transmission of a specific disease. Two guinea pigs of a total of four which had originally been inoculated with blood and bone marrow had shown irregular mild temperature spikes (not above 104°F). When these animals were sacrificed blindly (on days 9 and 19 post-inoculation) petechial hemorrhages into the peritoneum and, in one instance, into the pleura as well, were observed. This phenomenon was not seen in the companion guinea pigs inoculated with the same material nor was it reproduced on sub-passage in the next generation. Histopathological studies of tissues obtained from the two guinea pigs in question showed no specific pathological processes except sub-peritoneal hemorrhage.

Twice daily temperature readings on each guinea pig in this series of blind passages through five generations likewise failed to show any evidence of fever. The guinea pigs gained weight normally. In the 4th and 5th blind generations of two-passage lines, whose starting material was a blood-bone marrow inoculum, several unexplained guinea pig deaths occurred between the 5th and 9th day after inoculation. At first, the impression was that this phenomenon was reproducible but that not all of the inoculated guinea pigs succumbed. However, bacterial contamination of the inocula given the dying animals was shown in roughly one-half of the cases. In the remaining 50 percent, death could not be attributed to bacterial contamination.

Because these animals seldom showed any overt symptoms before their demise, due to the fact that deaths were sporadically distributed, these passage lines were temporarily suspended. As time and animal space permits, the most promising of these passage lines will be used to resume the experiment.

Five acute phase sera previously obtained and stored in the frozen state were inoculated into 5- to 6 day embryonated eggs by both the yolk sac and chorio-allantoic routes. Following the usual 20-30 percent inoculation mortality in the first 24 hours, an occasional egg died after 7-9 days incubation. Smears of the yolk sac of such eggs stained for rickettsia failed to demonstrate any organisms. Further egg passage of pools of yolk sac and embryo from such eggs failed to give any specific pattern of death or morbidity. Blind egg passages were not attempted.

Throat washings from patients in the acute stages of epidemic hemorrhagic fever were obtained in three instances. When treated in the usual manner with antibiotics, and inoculated into the extra-embryonic space, 9 to 10 day fertile eggs failed to show evidence of infection after incubation for five days at 37°C.

Standard isolation procedures when applied to all available laboratory animals had failed to yield an infectious agent for epidemic hemorrhagic fever. Prompted by the desirability of recovering a specific agent, efforts from this time on employed extraordinary techniques. In many instances, little precedent existed for some of the attempts; in others, isolated observations by other investigators suggested the use of their specific techniques. Two of the monkeys inoculated in experiments described above, each of which had been inoculated several times with material from patients with epidemic hemorrhagic fever, were splenectomized to determine whether so-called hyper-splenic resistance played any role in the suppression of that animal's infection. Each of these two monkeys at the time of operation had shown a rather abrupt rise in total leukocyte counts (20,000-22,000 from levels of 6,000-8,000) some 3 to 4 weeks after their last, and 10-12 weeks after their first, inoculation. The animals tolerated the procedure well and, except for instability of leukocyte counts for approximately two weeks post-operative, no significant disease was observed. The surgically removed spleens were passaged into normal monkeys without results. An attempt to passage material in splenectomized guinea pigs failed because of the high post-operative mortality.

Kilbourne and Horsfall (4) recently described the successful reduction of the resistance of adult mice to overt Coxsackie virus infections by pre-treating these animals with a single 5 mgm dose of cortisone. By this technique they were able to produce fatal infections not only with suckling mouse adapted virus but also with unadapted viruses (original isolation) in a host which was commonly considered to be completely resistant. An experiment was devised to determine whether cortisone treatment of mice and guinea pigs would render them more susceptible to infection with the agent of epidemic hemorrhagic fever. In this attempt, whole blood obtained from two patients within 48 hours after the onset of fever, was inoculated at the bedside into guinea pigs and mice. Material from one patient was inoculated into animals which had been pretreated with cortisone, guinea pigs each receiving 4 mgm per day and mice 1 mgm per day, for four days before the intraperitoneal inoculation of blood. The other blood was inoculated into animals which were started on cortisone at the time of inoculation.

Inoculated guinea pigs failed to show any signs of infection even though they were maintained on cortisone for 40 days. Most mice tolerated 1 mgm of cortisone daily for approximately 8-10 days, at which time symptoms consisting of ruffled fur, ocular discharge, and irritability appeared. Death was observed to occur 96 hours after appearance of symptoms if cortisone was continued. In this experiment, death occurred in both inoculated (EHF) and control cortisone-treated mice. When deaths occurred in the former group, viscera obtained at autopsy were sub-passaged intraperitoneally into additional cortisone-treated mice. Passage was maintained for three generations without any specific disease entity appearing. It would appear in retrospect that the dosage of cortisone employed may have been too large, and that before additional experiments along this line are initiated, it will be necessary to determine the maximum cortisone dosage tolerated by mice for long periods of time.

Recently, Casals (5) has established intracerebrally in mice a strain of Lansing poliomyelitis virus (MEF-1) which propagates to high titer in the central nervous system in suckling albino mice. This was done by serial blind passage of brain at a fixed time interval through a large number of generations. A similar technique of blind intraperitoneal passage of viscera was attempted in 14-16 gm mice inoculated with blood and bone marrow from a patient the epidemic hemorrhagic fever.

Two of the six originally inoculated mice were sacrificed arbitrarily on the 7th day after inoculation and emulsion of liver, kidney, and spleen were sub-inoculated into additional mice. Such blind passage has been carried through six generations. In the third generation, ascites was observed in two mice on the 16th post-inoculation day.

This observation has been repeated, ascites appearing between the 12th and the 18th day after inoculation in each of the succeeding three generations in a uniformity of inoculated mice. The typical ascitic fluid is bacteriologically, parasitologically, and mycologically sterile, and appears to be a transudate. No rickettsial-like organisms have been seen in organ-impression smears made from these animals. Mice developing ascites at no time show toxic symptoms.

At the present time nothing is known of the mechanism of the production of ascites in these mice. Efforts directed toward determining the significance of these observations are currently in progress, and the data obtained in further experiments will be presented at a later date.

From these efforts it is apparent that the usual laboratory techniques employed for the isolation of viruses and rickettsiae have not been successfully used to adapt the etiological agent for epidemic hemorrhagic fever to laboratory animals. Adaptation of this disease to experimental animals must be successful before any significant knowledge of the natural history, prevention, and specific therapy of this disease can be gained. It is therefore imperative that continued effort be made to recover the infectious agent.

Some of the described experiments must be repeated and new techniques, designed to alter the resistance of laboratory animals to infection, must be developed.

Laboratory Confirmation of Variola Infections - 1951 - The occurrence of smallpox in Korea during the winter and spring of 1951 was described elsewhere in this report.

With the appearance of this disease in a large troop population, it was necessary to standardize a routine for the most expeditious laboratory confirmation of clinically diagnosed variola. During the first six months of 1951 specimens from 31 suspected smallpox patients were received in this laboratory. Material submitted included clotted blood, spinal fluid, spleen, kidney, skin, and vesicular fluid or scabs. As in the experience of other laboratories (6) the specimen of choice from the standpoint of ease of collection and shipment, and the maximal recovery rate of variola virus, was found to be vesicular or pustular fluid. Virus in this material, collected upon a sterile cotton swab and sealed in a tube containing anhydrous phosphorus pentoxide (P_2O_5), remains viable without refrigeration for the time usually required to ship the specimen to the laboratory.

Methods - Specimens received in the laboratory were processed in the following manner. Tissues were emulsified as 20 percent suspensions in saline containing 1000 units of penicillin and 500 micrograms of streptomycin per cc. After appropriate incubation (60-90 minutes at room temperature) the suspension was centrifuged lightly and the supernatant liquid after being cultured for bacteria was used as the original inoculum. Blood clots were restored to their approximate original volume with physiological saline and treated similarly. Serum was usually inoculated directly and was always cultured simultaneously. Swabs of dessicated pustular or vesicular material were extracted with 1.25-3.0 cc of saline depending on the size of the specimen, and such extracts were treated in a fashion identical to those described above. In addition to intradermal injection of rabbits and inoculation of the sacrificed rabbit cornea, this material was introduced onto the chorioallantoic membrane of the developing chick embryo.

Paul's Test -Scarified rabbit cornea was observed ophthalmoscopically twice daily after inoculation. Small crateriform lesions along the lines of scarification were usually visible between 36 and 48 hours after inoculation if the test was positive. When such lesions presented themselves, the rabbit was sacrificed, the eye harvested and processed simultaneously with a control inoculated eye according to the method of Scott and Simon (7). The presence of epithelial hummocks and Guarnieri bodies in the bases of the necrotic craters confirmed the presence of variola virus. No effort was made to pass such agents from eye to eye in rabbits.

Rabbit Intradermal Test - This test, when positive, showed areas of indur-
ation at the inoculation site surrounded by erythema 1-2 cm in diameter. Reaction
was generally noted to appear between 48 and 72 hours after inoculation. Attempts
at further passage of any agent producing skin lesions in the rabbit were made.
Most authorities (6, 8, 9) agree that variola virus is very difficult to passage
in this manner, while vaccinia can be readily transferred.

Chorio-Allantoic Membrane Isolation - Embryonated eggs inoculated on the
chorio-allantoic membrane with the original material were incubated at 37°C. One
egg was harvested on the third day after inoculation and, if necessary, additional
eggs were examined on two succeeding days for the presence of focal necrosis in the
chorio-allantoic membrane.

When pock-like lesions were observed, all membranes inoculated with a given spec-
imen were pooled and ground in physiological saline. The emulsions were tested after
high speed centrifugation for their capacity to fix complement with hyperimmune vaccin-
ia serum. Preliminary reports were rendered on the basis of this complement fixation
test.

Serological Procedures: Materials and Methods - A commercial rabbit testicle
adapted strain of vaccinia virus was used for the purpose of preparing standard com-
plement fixing reagents.

Hyperimmune Serum - Parker's method (8) for the production of complement
fixing anti-vaccinial serum was used. Infected rabbit testicle, obtained at a time
when rabbit temperatures had returned to normal and local testicular reaction had sub-
sided, was emulsified as 10 percent suspension in saline. Supernatant liquids, ob-
tained by light centrifugation, were given intravenously to rabbits. Inoculations
were given three times each week, beginning with a 1 cc dose the first week, and in-
creasing by 1/2 cc increments each week thereafter for three weeks.

Serum with complement fixing titers of 1:256 were obtained by this technique even
though anaphylactic shock was encountered in inoculated rabbits during the latter part
of this immunization schedule. It has been suggested (10) that the inoculation of 5
percent instead of 10 percent tissue suspensions would have materially reduced the
incidence of this complicating phenomenon.

Antigen Preparations - Two types of complement fixing vaccinial antigens were
prepared. Supernatant liquids obtained by centrifuging infected rabbit testicle emul-
sions at 1500 rpm for ten minutes served as one of these. These antigens were origin-
ally 10 percent infected tissue by weight, and were stored at -50°C until used. The
second preparation was a 10 percent suspension of vaccinia infected chorio-allantoic
membrane. These were centrifuged lightly and the supernatant liquid alternately frozen
and thawed until flocculent precipitates occurred. These precipitates were sedimented
by centrifugation at 19,000 rpm for sixty minutes in the high speed attachment of an
international PR-1 refrigerator. The supernatant, stored at -50°C, served as the
antigen.

Results - Isolation of a member of the vaccinia-variola group was successful
in 11 of 35 attempts. The relative efficacy of the three isolation procedures simul-
taneously employed is indicated in Table XII.

All eleven isolates fixed complement in the presence of vaccinia hyperimmune rab-
bit serum to titers ranging from 1:16 to 1:256.

A comparison of the virus isolation results with clinical diagnoses in 14 cases at
the 3rd Station Hospital is shown in Table XIII. All cases of clinically diagnosed var-
iola except one were confirmed by isolation of virus.

Table XII. Variola Isolations: Methods for Recovery of Virus 1951

Specimens Received	Paul's Test Positive	Intradermal Skin Test Positive	Chorio-Allantoic Membrane Isolation	Total Isolates
35	4	7	11	11

Table XIII. Correlation of Variola Recovery with Clinical Diagnoses

Specimen	Patient	Confirmed Variola Isolate	Final Clinical Diagnosis
V1-208	W. F.	≠	Variola
V1-363	A. H.	-	Chicken Pox
V1-378	C. V.	≠	Variola
V1-418	W. A.	-	Secondary Syphilis
V1-454	P. R.	≠	Variola
V1-463	R. C.	≠	Variola
V1-486	R. H.	-	Varicella
V1-498	F	-	Varicella
V1-502	M. C.	-	Variola
V1-633	I. B.	≠	Variola
V1-1500	R. M.	≠	Variola
V1-1665	P. T.	-	Varicella
V1-1679	L. D.	-	Streptococcal Toxemia
V1-2755	K. S.	-	Vaccinia

While the preparations of a standard reagent was necessary to provide material with which to type viruses producing pocks on the chorio-allantoic membrane, it was hoped that these reagents might also be used to detect antibodies in the serum of patients. If this proved possible, perhaps confirmation of infection with members of this group of viruses could be made without employing tedious isolation procedures. Accordingly, in a preliminary experiment, sera obtained from three patients recently convalescent from smallpox were checked for complement fixing antibodies with vaccinia antigens. Antibody of low titer (1:8, 1:16, 1:16) was found in convalescent variola serum when tested against testicular antigens of vaccinia virus. In the one serum tested, using both testicular and chorio-allantoic membrane antigens, the complement fixation titers were identical. Serum from recently vaccinated (cowpox) individuals failed to fix complement with these two vaccinial antigens.

Although confirmation of smallpox by detection of complement fixing antibodies in convalescent serum is of little value in securing a rapid laboratory confirmation, it could conceivably lighten the isolation burden in epidemics. A serological approach could profitably be used, once variola virus had been established as the agent responsible for a given outbreak, to confirm additional cases. In a limited test series, serum from vaccinated (cowpox) individuals failed to fix complement with vaccinial antigens whereas convalescent variola serum did. The validity of this test must be strengthened by additional observation.

Pneumonitis in Kobe - During early February 1951, an outbreak of pneumonitis occurred at the 8th Station Hospital. The epidemic is described in the Epidemiology Section. Blood was drawn from patients during the acute phase of illness, serum was separated from the clot in Kobe, and both serum and clots were frozen for shipment to this laboratory. Throat washings from 14 patients were obtained in phosphage buffered saline (pH 8.2) and frozen in paraffin sealed jars. All specimens were shipped to Tokyo, refrigerated at -50 C.

Because of the physical limitations of the laboratory, and the complete intercirculation of air between it, the Blood Bank, and other laboratories in the 406th Medical General Laboratory it seemed advisable to isolate this project. Accordingly, all of the following procedures were done in temporary facilities in another part of Tokyo.

Both the blood clots and the throat washings were inoculated into 3-week old albino mice by both the intraperitoneal and the intracerebral routes. Mice showing any abnormal symptomatology were sacrificed, and brain or spleen and liver were taken for both passage and histopathological examination. Thirty-nine specimens were inoculated by this method and a total of 49 passages were made in mice of material obtained from the originally inoculated animals. No transmissable infectious agent was recovered by this technique.

Embryonated eggs were inoculated with blood clot emulsions or antibiotic-treated throat washings into the chorio-allantoic cavity and the yolk sac using material from patients of the group who, on clinical grounds, suggested the greatest possibility of yielding an isolate. Embryonic death, or sluggishness occurring 72 hours or later after inoculation, was the indication for sacrifice of the eggs. At harvest, allantoic fluid and yolk sac were taken; allantoic fluid was spot-tested for the presence of hemagglutinating agents, and yolk sacs were smeared, stained, and examined for elementary bodies. A total of 77 egg passages were made from the 14 originally inoculated specimens. No hemagglutinating agent was recovered. Elementary bodies characteristic of those described for the Psitt-LV group of viruses were not seen in smears of yolk sac. No infectious agent was recovered in embryonated eggs.

Complement fixation tests for psittacosis-lymphogranuloma venereum, typhus fever and Q-fever, as well as hemagglutination inhibition tests for influenza were done on paired sera from 20 patients in this outbreak. All tests were negative.

Tissues obtained from both normal and sick parakeets from the 8th Station Hospital were inoculated into mice and eggs with negative results.

Suspected Outbreak of Newcastle's Disease in Chickens - Newcastle's disease was reported as a highly contagious and fatal infection of poultry prevalent in the Far East as early as 1929, three years after its official recognition as an entity in England.

The appearance of an infectious disease of chickens in a flock maintained by an Air Force Post Exchange unit in Nagoya prompted attempts to isolate the etiologic agent. These fowl were first seen by a Japanese veterinarian and by representatives of the Japanese Government Experimental Station for Animal Hygiene, and later by military veterinarians. The former group reported that the etiologic agent of this outbreak has been isolated, and identified as Newcastle disease virus.

Acting on this information, specimens of tissue from disease chickens were obtained and shipped in the frozen state to this laboratory. Pools of liver, lung and spleen from three individual chickens were emulsified in saline which contained penicillin and streptomycin. Tissue emulsions were inoculated into the amniotic and allantoic cavities of 9-day old chick embryos. After 72 hours of incubation at 36°C allantoic and amniotic fluids were obtained at harvest and tested for their ability to agglutinate chick red cells. No hemagglutination was demonstrated, and embryos of the originally inoculated eggs were not hemorrhagic.

Segments of submitted avian intestine were checked for parasites by the Department of Medical Zoology of the 406th Medical General Laboratory. These tissue segments contained ova of two types of Coccidia and were infested with ascaris and capillaria.

Because there was no active disease occurring in the flock at the time these specimens were obtained the possibilities of confirming the Japanese diagnosis of Newcastle's disease were materially reduced. No virus was isolated. This could be explained by the disappearance, or masking, of the virus by immunity in the flock as the epidemic spent itself.

Attempts are currently being made in collaboration with the Nishigahara Laboratory to establish the identity of the isolated agent with standard American strains of Newcastle disease virus.

ORNITHOLOGY: A joint project by this laboratory and the Virus Commission of the Armed Forces Epidemiological Board begun in July 1949 has been continued. The original overall purposes for this study were two: to survey representative samples of all species of birds which inhabit the Japanese archipelago for antibody to Japanese B encephalitis virus, and to study the overall population patterns of native Asian birds. Observations, made in a limited number of birds in 1949, suggested that neutralizing substances to Japanese B encephalitis virus was present in certain of the species which had been collected. Because large numbers of birds of each species would have to be tested in order to collect significant data, the past 18 months have been spent in collecting serum samples from as many birds as possible. Bleeding and population pattern studies were conducted simultaneously.

Serological Survey of Wild Birds in Japan - From July 1950 through December 1951, serum was collected from 2510 individual birds representing a total of 172 species. All sera were obtained by bleeding freshly killed wild birds from the heart. It was found necessary to pool the sera obtained from many species of small birds in order to obtain samples adequate for testing. A total of 2069 sera were sent to Dr. Wm. McD. Hammon for antibody assay. Results are available from the tests performed on the first 881 samples. A serum was considered positive if three of five mice survived following intracerebral inoculation of a mixture of the test serum and 50 LD_{50} of Japanese B encephalitis virus. If the serum sample was adequate, positive sera were titrated for the amount of antibody present. Of 102 species tested, 44 were found to have representatives, which had neutralizing substances for Japanese B encephalitis virus in their serum. While some species had 60 percent of the members positive, the overall average of positive birds was 18.5 percent. Table XIV summarizes the results of the serological testing together with those of examining blood smears for avian parasites. Superficial comparison does not suggest an intimate correlation between the presence of antibody and the presence of parasites.

Recognizing the possibility of error in interpreting data compiled from a limited series of tests, the fact remains that substances neutralizing Japanese B encephalitis virus have been found in some birds of a wide variety of species, collected in all parts of Japan. Japanese investigators (11) have reported the isolation of this virus from the blood of the sparrows and gray starlings. While this laboratory has never confirmed these observations it would seem logical to assume that the protective capacity of positive serum was due to specific antibody in at least some of the cases. Until such time as both the natural and experimental infections of birds with Japanese B encephalitis virus can be studied, the significance of the accumulating survey data cannot be adequately evaluated.

Reports on the bird sera tested to date would seem to indicate that many individuals, irrespective of species, had some relationship with the virus.

An attempt was made to determine the relationships of ecological factors to the infection rate as indicated by the presence of neutralizing antibody. It should be borne in mind that such an analysis is subject to continual revision as more information becomes available, and has to be interpreted in the light of the fact that in many instances variables are continuously changing. For example, the carrying capacity of a habitat for any given species would vary through changes within the habitat itself.

From the available information, it would appear, superficially, that several common ecological factors are favored by the positive species. Many of these birds are permanent residents which commonly are found in brushy cut-over areas in Japan. Most of them nest in trees or shrubs, and build substantial nests. They commonly bring off their altricial young in July and August. Only a few positive species were colonial, and many were gregarious at certain seasons of the year.

Population Studies - During the past 12 months a total of 186 days were spent in the field in the vicinity of Tokyo. In this area, 96,430 birds of 151 species were observed. The counts were made in nine localities, touching upon all types of habitats in the Kanto Plain and the surrounding foothills. An attempt was made to balance the

Table XIV. Neutralizing Antibody and Red Cell Parasitization in Japanese Native Wild Birds

Species	Neutralizing Antibody JBE	Blood Parasites	Species	Neutralizing Antibody JBE	Blood Parasites
Jungle Crow	1/26	13/28	Narcissus Flycatcher	0/0	2/13
Carrion Crow	3/12	9/30	Great Eastern Reed Warbler	0/13	4/18
Blue Magpie	16/31	4/56	Brown Thrush	2/6	1/10
Japanese Jay	5/14	11/19	Dusky Thrush	7/17	3/31
Grey Starling	11/134	17/223	Pale Ouzel	1/1	1/1
Red-cheeked Myna	0/1	1/6	Red-bellied Rock Thrush	1/11	1/13
Tree Sparrow	10/68	6/117	Stonechat	0/0	1/11
Hawfinch	3/6	1/8	Green Woodpecker	0/3	1/3
Greenfinch	3/9	1/62	Brown Owlet	0/1	1/1
Meadow Bunting	4/7	2/39	Little Egret	0/16	2/24
Rustic Bunting	4/4	1/23	Cattle Egret	0/1	1/3
Skylark	3/12	3/69	Black-crowned Night Heron	13/43	1/54
Tree Pipit	1/2	2/10	Little Bittern	2/3	1/9
Pied Wagtail	5/13	4/41	Mallard	0/20	1/20
Japanese Wagtail	0/2	1/9	Teal	0/35	1/36
Grey Wagtail	0/1	2/18	Harlequin Duck	0/0	1/2
Great Tit	4/4	2/34	Eastern Turtle Dove	2/35	4/44
Varied Tit	1/1	3/8	Green Pigeon	0/1	1/1
Coal-tit	0/0	1/8	Australian Curlew	0/1	1/1
Bull-headed Shrike	2/8	4/33	Whimbrel	0/3	2/6
Thick-billed Shrike	0/1	3/5	Eastern Dunlin	2/10	1/11
Brown-eared Bulbul	8/26	3/41	Woodcock	1/1	1/2
Sumatran Brown Flycatcher	0/0	1/6	Latham's Snipe	0/0	1/6
Long-billed Ringed Plover	0/5	1/6	Little Tern	0/0	1/19
Black-tailed Gull	1/10	7/16	Herring Gull	0/4	1/8
Indian Moorhen	0/2	1/4	Bamboo Pheasant	1/10	1/12

areas observed so that relative bird population densities were not overshadowed by concentrations in certain localities. However, the concentration of water fowl at Shin Hama Duck Refuge tended to increase the importance of these species over terrestrial forms. Although less often seen, land forms were of equal abundance but were distributed over a wider area. Only 17 species were seen in abundance greater than one percent of the total and 31 in more than one-half percent. These were as follows: Jungle Crow .62 percent, Blue Magpie .55 percent, Great Tit 1.07 percent, Longtailed Tit .67 percent, Brown-eared Bulbul 1.00 percent, Eastern Great Reed Warbler 1.59 percent, Dusky Thrush .48 percent, House Swallow .98 percent, Plumed Egret 7.89 percent, Little Egret 4.63 percent, Black-crowned Night Heron 13.09 percent, Mallard 2.31 percent, Teal 13.32 percent, Shoveller 1.30 percent, Pochard 1.07 percent, Tufted Duck .89 percent, Golden Eye .67 percent, Japanese Cormorant 7.49 percent, Eastern Turtle Dove .59 percent, Asiatic Wandering Tattler 1.18 percent, Eastern Little Stint .82 percent, Kentish Plover 1.69 percent, Turnstone .7 percent, Asiatic Little Tern .59 percent and Black-tailed Gull 1.05 percent.

Each of these species followed a different population pattern. Table XV shows the ratio of observed individuals of a given species to the total observed bird population by month, the 31 tabulated species representing 89.5 percent of the total population. In this table each horizontal line of figures is essentially a graph of the species season population density. There has been no effort to break this data down by habitat. However, each species occupies its own group of habitats where its population pattern may be somewhat different from that indicated in the table.

Twenty-four percent of the birds bled in August 1950 showed neutralizing antibody to Japanese B encephalitis virus. Hypothetically, assuming that population tallies were accurate, for any given 10,000 birds in the Tokyo area in August, one would expect to find the following birds with antibody; 197 Gray Starlings, 642 Tree Sparrow, 37 Blue magpies, 517 Black-crowned Night Herons, 253 Plumed Egrets, 284 Cormorants, 2 Turtle Doves, 7 Skylarks, 180 House Swallows, 6 Jungle Crows, 110 Wandering Tattlers, and 244 individuals of other species.

During the late summer months, when the highest incidence of Japanese B encephalitis usually occurs, the 31 species listed in the table (XV) would be distributed in greater Tokyo as follows: Jungle Crows would be found in all of the habitats; the Blue Magpie would be limited to lowland wooded areas about farmhouses; the Gray Starling would be widely distributed traveling in flocks; the Tree Sparrow would be flocking in ripening grain fields and about farmyards. The small Greenfinch would be found in second growth forested areas. The Meadow Bunting would be among shrubs of woodland edges and in brushy cut-over areas. The Skylark would be found in cultivated fields and pastures. The Great Tit, the Long-tailed Tit and the Brown-eared Bulbul would be found in mountain forests. The Great Reed Warbler would be found in coastal reed beds. The House Swallow could be found about farmyards and city dwellings, raising final broods of young. Plumed Egrets, Little Egrets and Black-crowned Night Herons would occupy rice paddies and heron towns. Cormorants would be found in bays and estuaries and in rookeries. Turtle Doves would be widely distributed in fields and in wooded areas as well as about farmyards. The Wandering Tattler, Little Stint, Kentish Plover, Turnstone, Asiatic Little Tern and Black-tailed Gull would be arriving from the arctic and would be found on tidal flats and in rice paddies. The Rustic Bunting, Dusky Thrush, Mallard, Teal, Shoveller, Pochard, Tufted Duck and Golden Eye would not be present.

Each species has its own period of residency in the various habitats which it prefers from time to time during the year. In addition, it has a temporal relationship to the area of its range. This is shown for the 31 species listed in Table XV. The dates of arrival and of departure of migrant forms vary from year to year, but such variations are rarely great, and careful records kept during one year will usually establish the approximate dates.

Table XV. Population Variation in 31 Species of Native Japanese Wild Birds

Species	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jungle Crow	.62	.64	.90	1.17	.70	.82	.31	.57	.51	.48	2.59	.38	.72
Blue Magpie	.55	.31	.25	.97	.39	.96	.24	.55	.66	1.23	1.72	.37	.23
Grey Starling	5.86	7.94	1.18	6.28	.91*	4.02	4.08	6.62	11.81	9.04	1233	2.85	2.43
Tree Sparrow	14.83	9.01	10.35	14.29	5.54	13.96	1050	17.28	25.66	30.43	47.6*	6.69	5.70
Small Greenfinch	.94	.54	.40	3.20	1.01	.70	.31	.55	.34	.72	0*	2.05	2.41
Meadow Bunting	1.17	2.21	1.02	1.52	1.19	.85	.35	.51	.49	.54	1.33	1.32	3.34
Rustic Bunting	.79	.77	.68	3.78	2.08	--	--	--	--	--	--	1.01	3.82
Skylark	.84	1.14	.66	3.98	.88*	1.74	.77	.18	.46	.155	1.25	.45	.27
Great Tit	1.07	1.22	.87	1.91	1.37	1.14	.55	.5	.29	.86	3.22	1.70	2.15
Long-Tailed Tit	.67	.5	.13	3.35	0*	.20	.41	.07	.03	1.29	4.00	1.10	1.70
Brown-eared Bulbul	1.00	1.13	.55	189	1.84	.46	.18	.26	.29	.31	2.04	2.47	2.73
Great Reed Warbler	1.59	--	--	--	--	8.78	6.84	1.98	.21	.26	--	--	--
Dusky Thrush	.48	.78	1.3	4.25	3.59	--	--	--	--	--	--	.13	.22
House Swallow	.98	--	--	--	.18	1.65	1.48	2.09	2.05	1.68	1.17	--	--
Plumed Egret	7.89	--	--	--	.07	11.56	24.24	22.61	11.47	5.48	0*	--	--
Little Egret	4.63	1.65	.41*	2.77	.156	7.26	6.91	16.44	6.47	1.08	0*	.51	.74
Black-Crowned Night Heron	13.09	2.92	12.68	7.8	8.19	16.01	25.99	21.93	12.93	5.00	1.17	10.15	15.78
Mallard	2.31	1.13	19.7	.97	.78	--	--	--	--	--	0*	2.56	2.35
Teal	13.32	12.99	12.7	19.52	53.09	1.95	--	--	--	1.98	0*	43.44	28.72
Shoveller	1.3	.57	10.89	.19	3.95	.15	.03	--	--	--	--	.4	1.4
Pochard	1.07	1.54	6.97	1.95	--	--	--	--	--	--	--	.63	2.25
Tufted Duck	.89	.39	4.45	1.95	5.20	.03	--	--	--	--	--	.86	1.3
Golden Eye	.67	6.42	.03	--	--	--	--	--	--	--	--	0*	0*
Japanese Cormorant	7.49	30.0	1.79	.62	.44	.11	.01	2.32	4.96	18.24	9.03	5.94	8.3
Eastern Turtle Dove	.59	.77	1.04	1.05	1.01	.31	.33	.36	.33	.48	.47	.50	1.09
Wandering Tattler	1.18	--	--	--	--	5.85	.04	--	3.29	2.51	--	--	--
Little Stint	.82	--	--	--	--	3.74	--	--	1.43	3.75	--	--	--
Kentish Plover	1.69	4.25	0*	2.34	0*	.39	.68	.26	.58	2.93	0*	4.23	2.29
Turnstone	.7	--	--	--	--	3.78	--	--	2.49	.27	--	--	--
Asiatic Little Tern	.59	--	--	--	--	.93	1.03	0*	2.20	1.12	--	0*	--
Black-Tailed Gull	1.05	.26	.64	--	--	.03	--	.01	6.35	.19	.1	.02	--
Other Species	10.5	10.92	10.41	15.25	6.03	12.62	14.72	4.91	3.70	8.58	12.01	10.26	10.06

* Field data insufficient for accurate calculation

Extended Field Trips - During March and April and during October and November field trips were made to Hokkaido, northern Honshu and Kyushu. The objective of these trips was to collect birds and sera from widely separated geographical points of Japan to study the avi-faunal distribution geographically as well as by habitat and to follow migration patterns. The trips were planned to coincide with the major spring and fall migrations, and were made at the times when bird populations were most unstable.

In the spring, 128 species were observed, and 135 in the fall. The same general areas were surveyed on each trip. The impressions on bird populations gained from these studies are as follows: Some species such as Crows showed generally uniform populations in each locality and over the whole archipelago. Other species such as the Japanese Wagtail were present in one district and not in others. The movement of migratory species was evident, for flocks were intercepted moving north in the spring and south in the fall.

Overall populations varied considerably with the seasons and the area in Japan where observations were made. In the spring, immense flocks of migrating Gulls and Ducks were encountered in northern Hokkaido, and the average count was 500 birds per manhour of observation. In the fall, summer populations were moving out, and the count was only 21 birds per hour. In central Hokkaido the population was either more stable or the observations were made at times of nearly equal population density. The per hour count was similar both in spring and in fall. In northern Honshu the spring movement was delayed by weather, and only 20 birds per hour were seen. In the fall in the same area good weather was experienced, and a tally of 81 birds per hour was made. In central Honshu, a spring migration wave raised the count to 116 per hour. In this location in the fall, summer populations were still resident, were not augmented by migrants, and the count was 40 per hour. Observations were made in Kyushu at a time when many migrants had already moved north, and the count was 16 per hour. In the fall southern migrants were arriving, and the count was 37 per hour.

The avian population pattern of the Japanese archipelago as indicated by 18 months of field observations is essentially that of the deciduous forest biome and is subject to the changes to habitats brought about by the dense human population. Human pressure has reduced the avian population to a density considerably less than that found in similar North American habitats. Even though the bird population is low, the ecological niches are filled by different species of similar physiological needs to those in North America.

DEPARTMENT OF SEROLOGY

ROUTINE: Exclusive of Blood Bank activities and special projects, 112,423 routine procedures were completed in 1951. This total approximates that for the preceding year.

Table I. Routine Serologic Procedures

Cardiolipin Microflocculation Tests (Qualitative).............	80,126
Cardiolipin Microflocculation Tests (Quantitative)...........	9,078
Cardiolipin Complement-Fixation	13,188
Pandy	2,681
Colloidal Gold	2,670
Cold Agglutination	366
Heterophile Agglutination	2,273
Blood Grouping	379
Rh$_o$ Typing	368
Rh Antibody Titration	1,038
Miscellaneous	256

SEROLOGIC TESTS FOR SYPHILIS: In general STS showed a definitely lower proportion of doubtful reactions and slightly higher proportions of negative and positive reactions than in 1950. It was suspected that at least some of the troublesome doubtful flocculations might be due to departures from standard techniques. Deviations in antigen volumes, serum volumes, and mixing periods were investigated to determine whether reactions with sera of known reagin activity would be altered or remain unchanged. Varying the antigen volume from 1/10 to 1/90 cc. (standard 1/60 cc) without altering the serum volume (standard 0.05 cc) or the rotation time (standard 4 min.) had no appreciable effect on the results. Alteration of the saline and serum volumes from 0.03 to 0.1 cc and the rotation time from 4 to 6 minutes had no effect on reaction with 0.9% saline or with negative sera. Variation of serum volume from 0.03 to 0.07 cc. with variations of rotation time from 3 to 6 minutes for each serum volume, showed no change in reaction with positive and doubtful sera. Holding sera for 4 hours after inactivation without re-inactivation prior to testing showed little or no tendency to affect the results. Keeping antigen suspensions for 7 hours before mixing with sera likewise had little effect on the results of the test even when the serum was not re-inactivated prior to the test. However, delay of 4 minutes in reading the test resulted in a tendency for some tests to be read as more strongly positive than when the reaction was examined immediately after rotation. This tendency was increased when the delay was increased to 8 minutes.

The result of these investigations suggest that substantial departures from standard cardiolipin microflocculation test procedures (VDRL) do not cause significant changes in reactions. The only apparent exception to this finding involves excessive delay in reading reactions.

New color standards for reading the results of Wassermann tests were utilized in 1951. Two techniques were used, one for erythrocyte suspensions prepared daily and the other for DGV-preserved suspensions (see below). Both methods are summarized in Table II.

During the summer, 878 bloods from Ethiopian troops were surveyed for group and Rh antigens. From the results obtained it appears that the group and Rh distribution pattern is closest ethnically to the Negroid race. Findings are tabulated in Table III.

DGV SOLUTION: Preservation of sheep cells by DGV solution (dextrose-gelatin-veronal) was described in the 1950 Annual Report. Further investigation showed that pH deviations could be reasonably wide and that neither the DGV solution nor the cells needed to be fresh in order to prepare satisfactory suspensions. Such suspensions of preserved red blood cells were used for making color standards for the Wassermann test (see above) and for preserving the suspensions of sheep cells for complement fixation and heterophile agglutination tests.

Table II. Preparation of Color Standards for Wassermann Tests

FOR TESTS WITH CELLS IN

Reagents	Fresh Suspension				DGV-Preserved Suspension			
	Preparation A		Preparation B		Preparation A		Preparation B	
	ml.	%	ml.	%	ml.	%	ml.	%
Cell suspension	2.0	2	2.0	2	4.0 (sensitized)	1	4.0 (sensitized) Centrifuge, decant supernate completely	1
Distilled water	0		4.0 Mix thoroughly to obtain complete hemolysis		0		4.0 Mix thoroughly to obtain complete hemolysis	
Antigen	2.0		2.0		2.0		2.0	
Saline	6.0	0.85	2.0	2.55	4.0	0.85	4.0	1.7

Shake all tubes well. Prepare color standards as follows:

	1 ml.	2 ml.	3 ml.	4 ml.	5 ml.	6 ml.
Preparation A	2.5	1.87	1.25	0.63	0.31	0
Preparation B	0	0.63	1.25	1.87	2.19	2.5
Inactivated negative serum	0.2	0.2	0.2	0.2	0.2	0.2

Mix well and centrifuge

Color standards	4⁄	3⁄	2⁄	1⁄	⁄	Negative

Table III. Red Cell Antigens in Ethiopian Troops

Group	No.	o/o	Rh	No.	o/o
O	362	41.2	Pos.	347	95.9
			Neg.	15	4.1
A	250	28.5	Pos.	240	96.0
			Neg.	10	4.0
B	211	24.0	Pos.	201	95.3
			Neg.	10	4.7
AB	55	6.3	Pos.	53	96.4
			Neg.	2	3.6
Totals	878	100.0	Pos.	841	95.8
			Neg.	37	4.2

EPIDEMIC HEMORRHAGIC FEVER: The Serology Department was assigned the task of collecting convalescent serum from patients who had recovered from EHF. Maximum serum yield with minimum hemolysis was desired. After investigation, a gravity flow procedure, rather than the vacuum method of blood collection, was chosen. By the end of the year blood from 60 convalescent patients had been collected and set aside in a slanted position for harvesting (see Bacteriology Department report).

BLOOD BANK

The 8090th Blood Bank Laboratory Detachment, organized on 17 August 1950, continued to function until 5 November 1951, when it was replaced by the 48th Blood Bank Laboratory Detachment. Both units had the same mission which was to procure and process blood from donors in Japan. A separate section of the 406th Medical General Laboratory, the Blood Bank Storage Depot and Shipping Section, was responsible for receiving and storing blood obtained from the United States and from Japan and for distributing blood to medical installations in Japan and Korea. Although these were two separate units, their functions were so closely related that their activities are best described in a single report.

PROCUREMENT: Approximately 129,209 pints (76%) of the blood received by the Blood Bank Depot in 1951 was procured in the United States and the remaining 38,772 pints (24%) in Japan. Of the blood procured in Japan, 34.4 was contributed to the Blood Bank in Tokyo and 65.6% was collected by the Mobile Team at the various stations listed in Table I.

Table I. Mobile Team Collection Sites and Units Drawn

Units	No. Units Drawn	No. of Visits
37th AAA	203	2
40th AAA	471	6
ASA	291	3
Atsugi NAS	1565	6
COMNAVFE	402	4
Camp Drake	500	5
Camp Drew	293	3
Camp Ebisu (BCOF)	39	1
Camp McGill	3941	11
Camp Whittington	335	1
Camp Zama	2512	6
43rd Eng.	282	3
FEAMCOM	1304	4
Grant Heights	40	1
Haneda AFB	292	4
Japan Central Exchange	276	3
Japan Stockade	1275	10
Johnson AFB	369	5
Oppama NAF	150	1
Shiroi AFB	93	1
Tachikawa AFB	160	3
Yokota AFB	414	5
Tokyo Ordnance	354	5
TQMD	134	1
YED	441	6
Y Signal Depot	94	1
Yokosuka (U.S.N.)	6456	10
Yokohama Army Hospital	3308	
Total	25,994	

In Japan during the year 43,479 donors were interviewed and 39,597 pints of blood were collected through the combined efforts of the Central Blood Bank and the Mobile Team. Main reasons for rejection of donors were a history of: (1) Malaria; (2) Infectious Hepatitis; and (3) Hypertension. Only 172 cases (0.4%) of positive serology were encountered.

There were three periods of increased demand for blood in 1951. These increased needs were met during the February and June periods by mass appeals and increased donations in the Tokyo-Yokohama metropolitan areas and during the October period largely by increased military contributions.

The Blood Bank has received excellent cooperation from the American and Japanese Red Cross Organizations, AFRS Radio Tokyo, the Pacific Stars and Stripes, and other groups which have contributed greatly toward donation goals. Tape recordings of blood bank work were distributed in the United States for use by radio networks; documentary films of blood bank operations were made by the Army Signal Corps and Japanese studios for use both locally and in the United States; various posters, stories, and pictures were made for use by GHQ and JLC PIO's and for Stateside release; and a spectacular air rendezvous with the USS Boxer to obtain blood donations while the vessel was in action in Korean waters highlighted the year's activities.

A token shipment of blood was publicly received by General MacArthur from German employees of a world airline. The 4th of July saw the JLC Medical Section represented at the Meji Park Carnival by a working exhibit of complete blood bank operations and demonstration donors were processed in full view of the audience. A "Gallon Club" composed of donors who have contributed eight pints of blood to the U.N. effort was inaugurated in August. To date, there are 149 members of this honorary organization. In addition, letters of appreciation were sent to the Commanding Officers of 16 military installations who have supported the Mobile Team.

The Serology Department of the 406th Medical General Laboratory performed serology, typing, Rh and titre determinations on all blood procured in Japan. Table II shows the distribution of the various blood types. The distribution of blood types given in this table does not indicate a racial or geographical distribution. Many nationalities were represented among the donors, Americans predominating, and approximately 8.5% of the donors being Japanese Nationals.

Table II. Categorical Distribution of Blood Obtained in Japan

	Number	% For Each Blood Type
Type "O" (18,438 - 47.1%)		
Rh positive, high titer	10,378	56.2
Rh positive, low titer	5,142	29.7
Rh negative, high titer	1,848	10.0
Rh negative, low titer	1,070	5.9
Type "A" (14,560 - 37.2%)		
Rh positive	12,309	84.5
Rh negative	2,251	15.5
Type "B" (4,330 - 11%)		
Rh positive	3,722	85.9
Rh negative	608	14.1
Type "AB" (1,772 - 4.7%)		
Rh positive	1,511	85.2
Rh negative	261	14.8
Total		
Rh positive	33,062	84.5
Rh negative	6,038	15.5

DISTRIBUTION: The Blood Bank Storage Depot and Shipping Section was respon-
sible for distributing blood to the using agencies and for maintaining sufficient
stock on hand, generally a one week supply, in anticipation of all requests. Dur-
ing the early part of the year, there was considerable fluctuation in demand, often
with little warning of such fluctuation. Because of the tactical situation, such
changes could not always be anticipated. Since delivery of blood from the Z.I.
could not respond immediately to such changes, the basic policy was to maintain a
rather constant demand upon sources in the Z.I. and to alter collection in Japan
as required.

Blood was flown daily to sub-depots in Korea and Japan. As shown in Table III,
123,812 pints (72.5%) were shipped to Korea and 47,076 pints (27.5%) were shipped
to hospitals in Japan. Blood received from the Z.I. had an average of 12.9 usable
days remaining when received and 9.4 usable days remaining when shipped to Korea.
This information is shown by month in Table IV.

Table III. Summary of Blood Received and Shipped For The Year 1951

| | Received | | | | Shipped | |
	406th	ZI	Total	JLC	Korea	Total
January	2,614	7,064	9,678	6,293	4,989	11,282
February	4,233	9,264	13,497	5,402	9,178	14,580
March	2,319	9,224	11,543	3,733	9,629	13,362
April	2,952	13,466	16,418	2,296	12,108	14,404
May	3,287	15,032	18,319	4,689	13,366	18,055
June	3,053	10,528	13,581	2,933	10,368	13,301
July	2,454	10,392	12,846	4,081	9,624	13,705
August	1,926	9,048	10,974	2,113	8,112	10,225
September	2,722	11,496	14,218	4,570	9,864	14,434
October	5,769	14,424	20,193	6,150	14,056	20,206
November	3,871	10,632	14,503	2,517	12,482	14,999
December	3,572	8,639	12,211	2,299	10,036	12,335
Total	38,772	129,209	167,981	47,076	123,812	170,888

Balance on Hand 1 Jan 51 - 5,540
Balance on Hand 31 Dec 51 - 2,633

Table IV. Usable Days Remaining On Blood Received From Z.I. And
Shipped To Korea

Month	Received From Z.I.	Av. No. Usable Days	Shipped Korea	Av. No. Usable Days
January	7,064	9.5	4,989	7.1
February	9,264	13.6	9,178	12.4
March	9,224	13.9	9,629	10.2
April	13,466		12,108	10.0
May	15,032	12.3	13,366	10.3
June	10,528	13.5	10,368	9.3
July	10,392	13.5	9,624	7.1
August	10,974	12.0	8,112	8.2
September	11,496	13.5	9,960	9.5
October	14,424	12.6	14,046	9.2
November	10,632	13.8	11,676	9.9
December	8,639	10.1	9,138	9.3

During the early part of the year, when the fighting front in Korea was fluid, two blood depots were in use and both were located in the southern part of the Korean peninsula. As the front stabilized, new sub-depots were established further north. At the close of the year there were three depots in close support of the front and two supplying rear areas. Couriers accompanied all blood shipments to Korea to prevent any unnecessary delay enroute. Hospitals in the Tokyo-Yokohama and northern areas of Japan were supplied directly on request by the blood depot. Hospitals in southern Japan were supplied through two sub-depots located in Osaka and Fukuoka. Table V gives a summary by month to the main using agencies in Japan and Korea.

In October, 1951, two officers visited all blood using agencies in Japan and Korea including the medical installations drawing blood from sub-depots. The methods in use for ordering, shipping, distributing, maintaining refrigeration and stock levels, and the available records were inspected and reviewed. Where indicated, suggestions were made to improve the blood bank service. It was found that there was approximately a 3% reaction rate of which 1.4% was of the urticarial type. However, available records were generally inadequate and did not permit conclusions concerning moderate to severe transfusion reactions, (see Pathology report).

At the same time an investigation was made of the relation between the amount of blood ordered and transfused and the number of men wounded in action (use factor). It was found that blood substitutes, such as plasma and albumin, were used only infrequently, while the trend toward increasing use of whole blood noted during World War II had continued. Whole blood was used frequently. Other factors influenced the use of blood. During the early months of the war, because of the large number of casualties and the relative shortage of hospital beds, Japan was in effect a rear section of a battle zone, receiving patients who had been wounded only a few days previously. Later, when the tactical situation was stabilized, Japan was, in effect, a communication zone since hospitals in Korea worked with a 30- day evacuation policy. These changes in situation affected the use of blood.

Still another factor influencing the demand for blood was a result of communication and transportation difficulties. Medical units in Korea maintained stocks of blood in anticipation of high casualty rates and the possibility of being unable to replenish such stocks immediately. The hospital commander who had seen 100 pints of blood used in a single influx of casualties would not reduce his high stock level until assured that active combat had subsided and that the threat of a sudden return of combat activity had disappeared. Efforts were made to reduce stock levels in all blood depots during periods when enemy activity was light. Such decisions were always made with the realization that though excess amounts of blood might be undesirable, inadequate amounts would be disastrous.

Records of the actual amount of blood given generally were not available. The following use factors are therefore based on the amount supplied per wounded in action. This "use factor" has varied throughout the eighteen month old Korean campaign but, in general, it has tended to increase gradually. During World War II the Whole Blood Committee in the E.T.O. used approximately 0.2 pints per W.I.A. as a base estimate for supply planning purposes. The current campaign has shown a higher figure at all times. The early months of the Korean conflict revealed approximately 0.3 to 1.4 pints supplied per W.I.A. During the early part of 1951, 1.9 to 2.8 pints were supplied per W.I.A. and during the latter part of the year this figure increased to 4.9 pints per W.I.A. Average figures for the first 18 months of the Korean conflict are 1.8 pints supplied per W.I.A. in Korea and 0.9 pints per W.I.A. hospitalized in Japan. For both Japan and Korea, the average was 2.4 pints supplied per W.I.A.

SHIPPING AND BLOOD CONTAINERS: Containers used for shipping blood from the Z.I. were originally of heavy carboard which deteriorated with extreme rapidity under open storage and combat conditions. This made necessary the repacking of blood for Korea using a Navy type wooden box. The supply of this type container being inadequate, locally fabricated containers of that type were purchased and are still in use. Blood is now being shipped from the Z.I. in much better constructed containers which,

Table V. Summary Of Shipments To Main Using Agencies During The Year 1951

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Tokyo A.H.	583	738	903	640	737	726	748	504	593	933	535	321	7,961
Osaka A.H.	288	536	503	767	1007	998	697	760	1184	1792	902	788	10,222
8157 A.U.	2	2	20	2	9	9	11	4	1	9	14	3	86
8168 A.U.	130	166	43	138	119	78	42	55	115	107	96	122	1,211
8162 A.U.	1262	992	846	544	652	588	472	264	432	837	298	337	7,524
279 G.H.		96	224	16									336
J.A.B.				23	16	67	48	73	105	204	143	195	874
FEAMCOM							19	4	25	15	29	44	136
343 G.H.									23	186	208	44	461
Oper. "X"										44	179	2	225
6110 AFH											116	112	228
8165 A.U.												72	72
Yokosuka											6	24	30
Consolation	96		192		46							80	414
Repose	160												160
Haven	48	48	45	16					96	48			301
K-2	8	968	2640	4316	4716	7992	3096	192		24	•1416	832	23,952
K-9	4677	8162	6752	7776	5904	2064	1440	1224	1176	1224	8456	7440	42,647
K-14; K-16						312	5088	6696	8688	10686	2606	1652	47,366
S.K.						2064				2064			6,322
Totals	7,254	11,708	12,168	14,240	13,206	12,834	11,661	9,776	12,438	18,173	15,004	12,068	150,528

however, are used only between the Z.I. and Japan. They are being accounted for by count and also by serial numbers and periodic return shipments of the empty containers are made to the Z.I.

A comprehensive study of the various types of shipping containers was made in an effort to determine sturdiness, efficiency and duration of refrigeration, weight, and other pertinent factors that could be used as a guide for the local manufacture of these containers. The carboard type container was superior in refrigeration factors, largely due to the greater capacity of the ice container used. However, generally they could be used for only one shipment to Korea. The wooden containers, both Japanese and American, could be used for repeated shipments to Korea and maintained adequate temperatures for 20-38 hours, depending on the degree of exposure of the boxes to summer weather conditions.

A series of 100 units of blood were drawn into plastic bag containers which had been designed for field use. A control group of 100 units was also drawn into standard type Baxter bottles with ACD solution, and the two groups were compared for degree of hemolysis due to storage, handling, and transportation. A significantly lesser degree of hemolysis was noted in the Baxter bottle control group.

Fragility tests were performed on blood 8-10 days outdated and also on a group of bloods daily for 10 days beyond the 21 day expiration date to investigate the possibility of a 10 day extension of the expiration date in emergency situations. Both groups showed only a minimally increased cell fragility according to Wintrobe's standards for normal blood (without preservatives).

Department of Medical Zoology

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